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Weight status, heart rate variability and habitual physical activity in British Primary School children

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Abbreviations

HRV – Heart Rate Variability

PA – (Habitual) Physical Activity

BMI – Body Mass Index

SA – Sinoatrial node

RSA – Respiratory Frequency

ANS – Autonomic Nervous System

PNS – Parasympathetic Nervous System

SNS – Sympathetic Nervous System

SDRR or SDNN – Standard Deviation of RR intervals

RMSSD – Root Mean Squared of Successive Differences of RR intervals

NN50 – The number of pairs of successive R-R intervals that differ more than 50ms

pNN50 - The percentage of adjacent cycles of R-R intervals that are greater than 50ms

HF – High Frequency

LF – Low Frequency

VLF – Very Low Frequency

ULF – Ultra Low Frequency

CHD – Coronary Heart Disease

ECG – Electrocardiography

HRM – Heart Rate Monitors

TP – Total Power

FFT –Fast Fourier Transformation

AR –Autoregressive

ICC – Intra-class coefficient

IOTF – International Obesity Task Force

SPSS – Statistical Package for the Social Sciences

ANOVA/ ANCOVA – Analysis of Variance, Analysis of Covariance

Glossary of Heart Rate Variability Indices

Mean RR – The average differences in duration between successive R-R intervals

SDRR – Average standard deviation of all R-R intervals within a sample. Reflects overall variation in the heart beat series

RMSSD – Root mean square of successive differences in R-R intervals. Reflects the overall variation in the heart beat series

pNN50 – The percentage of adjacent cycles of R-R intervals that are greater than 50ms

HF – High frequency band (0.15 – 0.4Hz) driven mainly by the parasympathetic innervation of the heart

LF – Low frequency band (0.04 – 0.15Hz) driven mainly by the sympathetic innervation of the heart

VLF – very low frequency (0.0033 – 0.04Hz) influenced by thermoregulation and humoral system activity

ULF – Ultra low frequency (0 – 0.0033Hz) is related to circadian rhythm

Abstract

The purpose of this study was to examine the relationships between habitual physical activity (PA), weight status and heart rate variability (HRV) in British Primary School children. A total study population of 197 children (46% boys, 54% girls, mean age 9±1.4, 87% Caucasian, 11% Asian, 2% African-Caribbean) were recruited from a Coventry, UK primary school. Height and weight was measured to calculate BMI. Children were categorised in weight status (normal weight, and overweight/obese) using internationally accepted cut-off points. Heart rate variability was measured in the supine position for a 10 minute period, with the final 3 minute sample used for analysis in the time and frequency domain using Kubios HRV Pro (Kubios, Finland). Yamax Digiwalker SW800 (Yamax, Japan) pedometers were the chosen method of measuring habitual physical activity over a four day period (2 weekdays, 2 weekend days). Results from the study revealed a significant correlation between average step count and mean RR ($p=0.016$ $r=0.218$) and LF ($p=0.047$ $r= .180$) although no other significant correlations were observed ($p>0.05$). No significant weight status differences were found for HRV indices in the time and frequency domain ($p>0.05$), nor was weight status significantly correlated with average step count ($p=.959$). ANCOVAs revealed significant age differences for mean RR ($p=.014$), SDRR ($p=.05$), RMSSD ($p=.022$), but not for HRV indices in the frequency domain. In conclusion habitual physical in terms of average step count is a predictor of mean RR and LF in children independent of age. HRV indices are not influenced by weight status, and average step count does not influence weight status.

Key words: Heart rate variability, habitual physical activity, BMI, pedometers, weight status, primary school children

Chapter 1 Introduction

1.0 Background information

Heart Rate Variability (HRV) is defined as an index of tonic autonomic activity (Blom et al 2009) which in layman's terms is the fluctuations between consecutive heart beats. These fluctuations represent the changing levels of the activity of sympathetic and parasympathetic nerves, known collectively as the autonomic nerves, and modulate the intrinsic sinus node firing rate (Yutaka et al 1989). The normal rhythm of the heart is controlled by the membrane processes of the sinoatrial node (SA node) which are controlled by the divisions of the sympathetic and parasympathetic nerves of the autonomic system (ANS) (Berntson et al 1997). At rest the parasympathetic nerve systems dominates the heart rate (Goldberger et al 2001). HRV is a non-invasive tool that measures the autonomic control of the heart (Leicht, Sinclair and Spinks 2008), and has considerable potential to assess the role of autonomic system fluctuations in normal individuals and in patients with cardiovascular disorders (Task Force 1996).

The sympathetic influence on heart rate is mediated by release of epinephrine and norepinephrine, whilst the parasympathetic influence is mediated via release of acetylcholine by the vagus nerve (Task Force 1996). In addition to direct neural innervation of the heart, the sympathetic system can modulate heart rate indirectly through the release of adrenomedullary catecholamines (Bernston et al 1997). Other hormonal influences that may influence heart rate variability include variations in the activity of the renin-angiotensin system (Akselrod et al 1981). Under resting conditions the vagal tone succeeds and variations in heart period largely depend on vagal modulation (Levy 1971; Chess et al 1975). Parasympathetic influences exceed sympathetic influences through 2 mechanisms: 1) a cholinergically induced reduction of norepinephrine released in response to sympathetic activity and 2) a cholinergic attenuation of the response to adrenergic stimulus (Task Force 1996). The parasympathetic nervous system is able to modulate heart rate effectively at al frequencies between 0 and 0.5Hz, whereas the sympathetic nervous system modulates heart rate with significant gain only below 0.1Hz (Bernston et al 1997). Circadian HRV reflects a wide range of determinants, including changes in activity, posture, breathing, autonomic outflow, state of arousal and a range of behavioural variables (Bernston et al 1997).

The R-R interval variations present during resting conditions represent a fine tuning of the beat-to-beat control mechanisms (Akselrod et al 1985). Vagal afferent stimulation leads to reflex excitation of vagal efferent activity and inhibition of sympathetic efferent activity (Schwartz et al 1973). The opposite reflex effects are mediated by the stimulation of sympathetic afferent activity. Efferent sympathetic and vagal activities directed to the sinus node are characterised by discharge largely synchronous with each cardiac cycle that can be modulated by central (vasomotor and respiratory centers) and peripheral (oscillation in arterial pressure and respiratory movements) oscillators (Malliani et al 1991). These oscillators generate rhythmic fluctuations in efferent neural discharge that manifest as short- and long-term oscillation in R-R intervals

Periodic components of HRV tend to be combined within several frequency bands, for example the respiratory frequency (RSA). The RSA band is considered to range from about 0.15Hz to 0.4Hz in humans but may extend below 0.15Hz and above 1Hz in infants. RSA is mediated by vagal-cardiac nerve traffic fluctuations and thus may be an indicator of vagal activity. R-R interval oscillations occur at low frequencies (0.05-0.15Hz) and termed the Low frequency band (LF). Other R-R oscillations occur at frequencies below 0.05Hz commonly termed as ‘very low’ frequencies (VLF; 0.003-0.05Hz) and slower, ‘ultra-low’ frequencies

(ULF) (Bernston et al 1997). The RSA peak is at approximately 0.25 Hz at rest (with a range of 0.13- 0.33Hz), equivalent to number of breaths (8- 20 breaths/min^{-1}). The pulmonary centre in the brain stem modulates vagal activity to the lungs as well as to the heart during inspiration to cause bronchodilation and facilitate breathing (Freeman et al 2006). This results in an increase in heart rate during inspiration, which results in a high frequency (~12 times per minute) oscillation in heart rate, which in part may be due to stimulation of receptors in the lower airways by an increase in the volume of the lungs (Jordan and Marshall 1995)

Reduced beat-to-beat variability is a predictor of morbidity, mortality and arrhythmic complications and is found in individuals with a variety of cardiac abnormalities (Bigger et al 2007). The risk of mortality has been proposed to be significantly greater in individuals with HRV less than 50ms between successive RR intervals (Kleiger et al; Martin et al 1987). A reduced HRV positively correlates with obesity (Nagai et al 2004), poor aerobic fitness (Aubert et al 2001), low habitual physical activity (Blom et al 2009), and increasing age (Silvetti et al 2001), as well as racial differences (Reed et al 2006). However, although the literature on HRV contains a lot of research, it has mainly focused on the adult population while little research exists for children thus more research is required to determine whether factors such as obesity, habitual physical activity, age and ethnicity effect HRV in the same way in children as in adults.

Given that Coronary Heart Disease (CHD) is a major cause of mortality and morbidity in the UK with one in five male deaths and one in eight female deaths, totally around 88,000 deaths, with a further 43,000 deaths caused by stokes and 60,000 deaths from circulatory disease (British Heart Foundation 2010), with the disease process showing in children (Anderson et al 2003) it is important for scientists and public health practitioners to understand the relationships between physical activity and indices related to cardiac aliments such as HRV. Furthermore rising overweight and obesity levels have been associated with increased mortality due to an augmented risk of developing an impaired glucose tolerance, hypertension, fatty liver disease and CHD in both adults and children (Andersen et al 2003; Rudolph 2004). It is now known that CHD is partly a paediatric problem, in that the onset of CHD lies in early childhood or though the clinical symptoms of this do not become apparent until much later in life (Froberg and Andersen 2005). Physical activity has been highlighted as a potential positive influence in reducing CHD risk factors and obesity, and has been shown to positively affect HRV indices in adults (Hautala et al 2010) but there is a dearth of information on the relationship between HRV, weight status and physical activity in children, despite previous suggestions that this area should be further researched (Welk, Corbin, and Dale 2000; Nagai and Moritani 2004; Blom et al 2009). Investigation of this area may have important public health applications including effective targeting of interventions to positively influence HRV, weight status and/or PA in children.

2.0 Current body of knowledge

2.1 Measurements of HRV

In 1996, the European and North American Task Force released guidelines for the measurement, physiological interpretation and clinical use. Heart rate has conventionally been measured through the use of the electrocardiogram (ECG) and is deemed the 'gold standard' for measuring HRV, which requires mathematical algorithms in order to locate the peak of R-waves, and thus measure R-R intervals. However, more recently the development of wireless heart rate monitors has allowed for the exposure of R-R intervals to be detected. Furthermore, as heart rate monitors store heart rate data, this information can be downloaded onto specific software packages such as Kubios, allowing for analysis of HRV indices without manual calculations usually needed when converting ECG recordings into HRV measures. Several studies have therefore investigated the validity and reliability of such heart rate monitors in analysing HRV (mainly the Polar S810), all of which have concluded that specifically the Polar S810 heart rate monitor was a valid and reliable measure of R-R intervals and subsequent HRV analysis in the supine position for adults (Gamelin, Berthoin, and Bosquet 2006; Nunan et al 2009).

HRV measures are split into two categories; time domain indices, and frequency domain indices. There are two main groups of time domain variable measures (Stein and Kleiger 1999). The first of which include; mean R-R intervals (the differences between successive R-R intervals) and SDRR (average standard deviation of all R-R intervals), which reflect total power (Stein and Kleiger 1999). The second group includes pNN50 (the percentage of adjacent cycles that are greater than 50ms apart) and RMSSD (the root mean square of successive differences) which have been found to reflect vagal modulation of the SA node and correlate strongly with the high frequency (HF) power band (Pagani et al. 1986).

The frequency domain indices represent power density of HRV and provide information on the distribution of power as a frequency band; formed of both the high frequency band and the low frequency band. The HF (above 0.15Hz) frequency reflects respiratory mediated HRV, while LF (0.04 to 0.15Hz) is modulated by both the sympathetic and parasympathetic nervous systems (Stein and Kleiger 1999). In addition to HF and LF, frequency domain measures also include very low frequency (VLF) and ultra-low frequency (ULF), although the underlying physiological mechanisms involved are less distinguished than HF and LF (Task Force 1996).

There are standardised durations for the recordings of HRV, with nominal 24 hour long recording and short-term 5 minute recordings. The 24-hour standard deviation of normal R-R intervals (SDNN) provides a simple and sensitive scale of cardiovascular risk, while the spectral analysis of HRV over 5 minutes helps to quantify the sympathetic-vagal balance alterations which play an important role in the detection of cardiovascular disease (Malliani 1999). More recently, 3 minute segments of R-R recordings have been used (Fukuba et al 2009; Weippert et al 2010) which are appropriate to assess HF and LF as the minimal amount of time required for detection is 2 minutes (Task Force 1996).

The position or posture of the body impacts the sympathetic and parasympathetic modulation of the heart. Literature suggests that adjustments to heart rate modulation from the supine to the sitting posture are due to hydrostatic changes caused by the displacement of blood from the central region to the lower extremities, thus reducing cardiac debt, systemic arterial pressure and activation of the arterial and cardiopulmonary receptors (Lindqvist 1990; Acharya et al 2005).

There is a considerable amount of reliability and reproducibility studies of HRV in adults although these have not been conclusive as determined by a review by Sandercock et al (2005). Freed et al (1994), Sinnreich et al (1998), and Marks and Lightfoot (1999) have all found HRV is a moderately reliable measurement from stable electrocardiography (ECG) recorded under controlled, resting conditions. Within individual resting measures of HRV have been found to be reliable including; RMSSD (r = 0.20–0.98), pNN50 (r =0.43–0.97) HF (r = 0.48–0.96), LF (r = 0.60–0.97), and TP (r = 0.52–0.97) (Hohnloser et al 1992; Pitzalis et al 1996; Melanson 2000). The design of the above studies range in terms of the re-test period time with Pitzalis et al taking a baseline 10 minute ECG measurement, and re-testing after 2 weeks and again 7 months later, measuring in 3 conditions of rest, controlled respiration and passive head-up tilt. Hohnloser et al took Holter recordings on days 1, 7 and 28 at rest comparing normal subjects against subjects with cardiac disease. Whilst Melanson took resting ECG measurements over 5 consecutive mornings in 37 men after an overnight fast and before any strenuous exercise. However, Sandercock et al (2005) concluded that further research is required to accurately assess the reliability of HRV. Furthermore, LF was reproducible under all three conditions of rest, tilt and controlled respiration, total power was reproducible at rest and HF only during controlled respiration. Therefore, the reproducibility of the frequency domain parameters depends on the analysed condition (Pitzalis et al 1996).

However, in terms of children there have been very few such studies looking at reproducibility (test-retest reliability) which is important in terms of interpretation of research as reliable results for adults may not generalise in younger individuals (Dietrich et al 2010) due to the age-dependent differences in autonomic functioning (Tanaka et al 1998). One of the first to precisely investigate the reliability of HRV measures at rest in children was Winsley et al (2003) in which a sample of 12 children, ages 11-12 years old HRV was measured for a period of 5 minutes in a supine position, with breathing controlled at 12 breaths/minute. The outcome of this study was to suggest that HRV measures at rest in children are unreliable. However, this study has limitations in its sample size of only 12 children and using just one age range of 11-12 years old. Furthermore, controlling the breathing rate for children may have led to the unreliability of measures because it may have led to the children becoming anxious as they attempt to maintain the 12 breaths/minute, thus affecting the resting heart rate of the children. The authors of this study concluded that more research was required in this area to which led Dietrich et al (2010) to conduct a study on 57 children ranging from the ages of 10-13 years old, HRV was measured by ECG in both the supine and standing position for a period of 5 minutes, and found to be moderate-to-high in terms of reproducibility according to the magnitude of relative and absolute reliability. The authors of this study conclude that relative reliability of short term measurements of HRV in the time and frequency domains may be considered satisfactory in the supine and standing positions in children, thus implicating a significant capacity to detect real differences between children.

2.2 Age, sex and race differences in HRV

Age and gender significantly affect heart rate and various components of HRV in the supine position and in response to standing (Fagard, Pardaens and Staessen 1999). The increase in HRV and respectively the decrease in heart rate through childhood have been related to the growth of cardiac mass and stroke volume (Kmit and Rubleva 2001). Measures of HRV (Mean RR, SDNN, RMSSD, TF, VLF and HF) in children aged 6-16 years old increase with age in a wavelike way changing from year to year, although it is believed that HR control is stabilised between 15-16 years old as no differences were observed (Galev, Igisheva, and Kazin 2002). The term ‘wavelike way’ used by Galev et al (2002) refers to the varying age differences observed in HRV parameters, in that the maximal SDNN was observed in children aged 9, 13, and 14 with the minimal values observed in children aged 7 and 12. Similar aged differences were found in RMSSD with the maximal median observed in ages 9 and 13, and minimal values in 7, 8 and 12 year olds, thus indicating that the age differences in HRV parameters do not increase in a controlled linear fashion. This is supported in research that has found that overall HRV measures increased with age and were unrelated to gender, and also indices of parasympathetic function increased up to the age of 10, again gender-unrelated (Silvetti, Drago, and Ragonese 2001). HRV parameters are known to decrease with an associated increase in age (Bonnemeier et al 2003). The effect of aging on HRV is linear up to the age of 60 years; thereafter the age-related decrease in HRV stabilizes

(Koskinen et al 2009). With aging decreased heart rate responses to β-adrenergic agonists are seen (Kendall et al 1982), heart rate responses to β-adrenergic blockade are also blunted (Pfiefer et al 1983), and parasympathetic mediated respiratory variation in heart rate decreases with aging, and muscarinic parasympathetic blockade with atropine results in lesser increases in healthy elderly vs younger individuals (Pfiefer et al 1983). Furthermore, the plasma concentration of the noradrenaline, as well as efferent sympathetic nerve traffic to skeletal muscle increases with age (Stolarz et al 2003).

Several studies have investigated sex differences in the measures of HRV, with the evidence somewhat divisive with certain studies suggesting that females have a higher HRV than in males (Evans et al 2001; Koskinen et al 2009). However, other researchers have found the opposite, suggesting that males have a higher HRV than females (Umetani et al 1998; Bonnemeier et al 2003). There is also evidence to suggest that there is no sex difference in HRV (Sloan et al 2008). Bonnemeier et al (2003) however, stated that the significant sex differences decrease with aging. Furthermore to studies investigating sex differences in the adult population, studies have also investigated whether sex differences in HRV are observable in children. Research again is conflicting with certain studies suggesting that there is an absence of HRV differences related to sex (Reed et al 2006; Krishnan et al 2009), although it is suggested that differences between boys and girls in HRV parameters (SDNN, RMSSD and HF) are observable from 12 years of age but gender differences were observed as early as 9 years of age in parameter M (mean RR) (Galev, Igisheva, and Kazin 2002). However, it has been found that overall measures of HRV are significantly higher in boys than in girls across all ages measured (years 1-20) (Silvetti, Drago, and Ragonese 2001). The mechanisms underlying age-related gender differences in HRV are undistinguished, but has been suggested there are lower levels of parasympathetic activity in young females, which declines (above the age of 30) compared to more stable levels of sympathetic activity (Umetani et al 1998).

Another variable that may have an effect on HRV in individuals is race or ethnicity. Firstly, Liao et al (1995) reported that African Americans had a lower LF, higher HF, and a larger HF: LF ratio than Caucasians. These findings have been found in further research, suggesting that both in adults and adolescents, African American individuals have lower measures of HRV although mainly measured in the frequency domain (Guzzetti et al 2000; Faulkner, Hathaway and Tolley 2003; Zion et al 2003). This has been suggested to be due to parasympathetic activity decreasing progressively with aging only in Caucasians and not in African Americans, with African Americans having a lower parasympathetic activity comparable to similar aged Caucasian populations, which is reflected by HF and LF measures of HRV (Choi et al 2006). However, there is evidence to suggest that African Americans have higher resting HRV than European Americans (Wang et al 2005; Li et al 2009). With all the research focusing on the comparison between African Americans with Caucasian, Reed et al (2006) were the first to examine differences between Asian and Caucasian children within a Canadian community following research that suggested there may be differences between Asian and Caucasian individual, although no studies had compared the two ethnicities. The results suggest that Asian children had a higher HF: LF ratio than Caucasian children of the same age. However, although not significantly higher, Caucasian children registered higher time domain measures (SDNN, RMSSD) in the supine position than Asian children.

2.3 Weight status and HRV

Childhood obesity is becoming a major epidemic concerning the health and wellbeing of children, with the acute effects of obesity on children being an increase in the likelihood of developing an impaired glucose tolerance, hypertension and fatty liver disease (Rudolph

2004). Furthermore, the greatest health problems occur when children move into adulthood with obesity-related illnesses such as diabetes, cancers, gall bladder disease, endocrine disorders and osteoarthritis (Lobstein et al 2004). Previous data suggests that the sympathetic nervous system (SNS) is important in the regulation of an individual's energy balance, and altered SNS activity may affect the amount of fat mass in humans (Acheson et al 1988; Spiegelman and Flier 2001). The effect of an increased BMI of an individual on the cardiovascular system contains literature to suggest that it is associated with a reduced HRV caused by a withdrawal of the parasympathetic tone and/or the increase in sympathetic modulation, attributed to the decreased adreno-receptor responsiveness, vagal withdrawal and/or increased sympathetic activity (Ramaeker et al 1998; Fraley et al 2005; Schmid et al 2010). In addition, changes in weight status in either direction have been associated with changes in HRV (Hirsch et al 1991; Poirier et al 2003). More recently the possible impact of adipokines such as leptin on the autonomic nervous system has been hypothesised, with a relationship between concentrations of leptin and resistin in the blood and heart rate variation parameters observed indicating a possible link between adipokines and the disturbance of the autonomic nervous system (Piestrzeniewicz et al 2008). Paolisso et al (2000) found that leptin stimulated the sympathetic nervous system, thus increasing heart rate and arterial pressure. Furthermore individuals who are obese and/or hyperinsulinemic have increased adipocyte production of inflammatory markers, including CRP, IL-6, and TNF-alpha which are related to cardiovascular disease (Kasapis and Thompson 2005).

Previous studies of obese children and the effect on autonomic activity and HRV have produced inconsistent results. It has been found that obese children exhibit higher sympathetic activity and a reduction of parasympathetic activity with a withdrawal of vagal activity in comparison to normal weight children (Karason et al 1999; Sekine et al 2001).

This is reflected in HRV measures, with lower RMSSD, SDRR and HF and an increased LF: HF ratio (Martini et al 2001; Rabbai et al 2003). However, more recent studies have concluded that obese children have both a reduced sympathetic and parasympathetic tone (Nagai et al 2003; Vanderlei et al 2010). Furthermore, there is research to suggest there is no change in sympathetic activity but a reduction/hypoactivity of the parasympathetic tone linked to RMSSD and LF (Yakinci et al 2000; Kim et al 2005). In addition, Rabbia et al (2003) found that children who were obese less than 4 years had significantly increased sympathetic activity, but children who were obese for more than 4 years were no different to healthy individuals suggesting that the period of obesity may be an important variable to consider when evaluating obesity and HRV.

2.4 Physical Activity children

It is now a widespread belief that most people in the developed world are not meeting current guidelines for physical activity attributed to the increase in pleasurable sedentary activities such as T.V. and especially for children video games (Buchhiet et al 2007).

2.4.1 Activity patterns of children

The lack of appreciation, and quantification of the transitory nature, in terms of the short durations of activity patterns in children may have affected the ability of studies to provide an accurate reflection of children's activity and this will have impacted on the determination of associations between activity and health (Rowlands and Eston 2007). Some researchers have investigated the intensity and duration spent in each bout of activity in children (Bailey et al 1995; Berman et al 1998; Baquet et al 2007). Bailey et al (1995) was the first to research in this area using direct observation of fifteen 6-10 year old Americans, and found that the median duration of low to medium intense activity was 6 seconds, and just 3 seconds for high intensity activities. The same data-set was re-assessed with spectral analysis by Berman et al (1998) which showed 83±11 bouts of activity per hour in boys and 89±12 bouts of activity in girls with a mean bout duration of 21±5 seconds for boys and 20±4 seconds for girls. More recently, Baquet et al (2007) used accelerometry monitoring and despite a change in methodologies, the reported results were similar to those reported from the observation technique. Mean bout duration in French 8-10 year old children was 22.1±3.5 seconds, and that 80, 93, 96% of activity bouts of moderate, vigorous and very high intensity respectively were shorter than 10 seconds. Although the duration of activity spent in vigorous and very high intensity was less than 3% it accounted for over a third of the total PA (Baquet et al 2007).

2.4.2 Increasing PA in children

There have been calls for the development of effective intervention strategies in order to promote and increase PA levels in children and adolescents to combat the increasing levels of obesity and improve other aspects of health (Department of Health 2005). A review paper by Van Sluijs et al (2007) examined the effectiveness of intervention studies to increase PA in children and adolescents, which found that in some interventions there were achievements of important changes such as a 13% increase in playtime spent in moderate to vigorous PA which has been suggested to be more beneficial in achieving improved HRV indices Buchheit et al 2005; Buchheit et al 2007). However, it was concluded that there is limited evidence of effectiveness of environmental interventions and those aimed at children from a low socioeconomic groups, although there is strong evidence for school based interventions including family and the community involvement and multi-component interventions in the effectiveness of promoting and increasing the level of PA on children. The lack of clear evidence for effective interventions was put down to a lack of high quality evaluations made by the papers reviewed especially in young children. Despite this, there have been studies using pedometers as a motivational tool to increase PA in children which have shown that rewards based on access to television viewing combined with pedometer-based goals are an effective way of increasing children's physical activity levels (Goldfield et al 2000; Roemmich et al 2004), but pedometer-based goals are not effective without a reward (Goldfield et al 2006).

2.5 Physical Activity and HRV in adults and children

The decrease in habitual physical activity (PA) has been suggested as a causality of increases in cardiovascular risk (Kohl 2001), and low habitual PA has been found to lead to a decrease in HRV (Buchheit et al 2007; Dietrich et al 2008; Blom et al 2009). In turn regular physical activity has beneficial effects on the autonomic control of the heart accompanied with an increase in HRV (Dietrich et al 2008) and is a predictor of LF, HF and SDNN (Blom et al 2009). Regular PA is partially associated with increased vagal modulation, linked to increased HF power (Hull et al 1994) and moderate-to-high PA results in higher parasympathetic indices/ outflow (Melanson 2000; Bucheit et al 2004). Most of the research into habitual PA and the relationship with HRV has focussed around the adult population despite it being said that identified cardiovascular risk factors may be found in early childhood or adolescence (Buchhiet et al 2007), thus provide an interesting and important area of research.

However, a few studies have been conducted with paediatric samples, although these studies are equivocal in findings. For example Blom et al (2009) examined cardiovascular risk factors using HRV and self-report measures of PA in 71 children aged 15years 11 months- 17 years 7 months (mean age 16.5 years) following exclusion for diabetes, thyroid dysfunction and more than 5% missing or distorted data from HRV measures. HRV was recorded using an ECG attached to the wrist during 2min x 2 periods with subjects sitting in an upright position with caffeine controlled 1 hour before testing and PA was measured on a 5 point scale by the frequency of exercise with hard breathing and sweating (“never”, “seldom”, “once a week”, “twice a week” and “more than twice a week”). The main findings of this study was that self-reported PA was a predictor of HRV measures (HF, LF and SDNN) in adolescents, thus suggesting lifestyle factors influence HRV at an age preceding the development of lifestyle-related cardiovascular disorders. However, this study uses the term exercise, and states that it is this rather than habitual PA that is a predictor/beneficial to HRV. Also Blom et al do not define “hard breathing or sweating” presumably leaving it for the subject’s interpretation, leading to either an over or under estimation of PA. It also does not measure for how long these periods of hard breathing or sweating take place for. The use of sweating as a measure of PA has been suggested to be limited in distinguishing active from inactive subjects in epidemiological surveys by Washburn et al (1990) thus leaves questions into the justification of the authors of these studies to use such a measure of PA.

According to Bucheit et al (2007) they were the first to investigate the incidence of habitual PA on both cardiorespiratory fitness and autonomic function as inferred from HRV in preadolescents. This study involved 67 12-year old children, with habitual PA assessed by a triaxial accelerometer and HRV recorded over a 15 minute period using a Polar 810s heart rate monitor. The authors reported that participation in activities for a period of 1 hour per week of higher intensity is necessary to observe favourable associations with vagal related HF and LF: HF ratio, whilst individuals who participate in moderately intense activities for a period of 210 minutes per week have higher physical fitness parameters such as VO_{2max}, HR recovery and muscular strength.

Furthermore, Krishnan et al (2009) followed up the above study by Bucheit et al (2007) examining the relationship between heart rate control and adiposity in 208 young children (aged 9±0.25 years). Again habitual PA was assessed by the use of accelerometers with PA categorised as low (e.g., sitting in class and watching television, 1000 c.p.m.), medium (e.g., walking and playing indoors, 1000–2500 c.p.m.), and high-intensity (e.g. running, skipping, jumping, and football, .2500 c.p.m.). HRV was recorded over a 10 minute period by use of an ECG and analysed in the time and frequency domain. The findings from this study are that resting HR correlates negatively with PA in boys but not for girls, and also in terms of SDNN and RMSSD but again only evident in boys.

2.5.1 Intervention studies to increase HRV

Regular PA/exercise has been associated with an increased HRV (Jurca et al 2004; Dietrich et al 2008), and as such forms a basis for many intervention studies looking to increase HRV in certain populations. It is well documented that long-term endurance training has a beneficial effect on cardiac modulation of the heart with increases in HRV, parasympathetic activity, and a decrease in the sympathetic activity of the heart at rest (Shi et al 2005; Gregoire et al 1996; Chen and Dicarlo 1998). Training intervention studies however are inconsistent in that studies in middle-aged participants have shown an increase in parasympathetic tone after aerobic training (Melanson and Freedson 2001; Amano et al 2001; Jurca et al 2004), whilst other studies show no improvement/effect on HRV following training (Boutcher and Stein 1995). Although it is suggested that moderate to high intensity physical activity or exercise has a greater effect on vagal related indexes of HRV (Buchheit et al 2005; Buchheit et al 2007). However, in a recent review, it is suggested that exercise therapy may improve HRV in myocardial infarction, and chronic heart failure patients from resulting increased vagal tone and decreased sympathetic activity (Routledge et al 2010).

Several studies have examined the effect of different exercise intensities on children, however again the research is inconsistent. Following a 13 week endurance training program (3×1 h week^{-1}; intensity > 80% HR_{max}) it was found that endurance training has a positive effect on nocturnal global HRV without changes in parasympathetic or sympathetic activity (Mandigout et al 2002). Negai et al (2004) followed this study with a 12 month school-based exercise program (20mins/day, 5days/ week, 130-140bpm) in 300 children aged 6-11 with results suggesting that moderate exercise training has a positive effect on cardiac ANS activity in children with initially low HRV. However, a 7 week high intensity intermittent training program in 52 children aged 9 years found that there was no significant effect on the autonomic control of the heart, measured by HRV despite improvements in aerobic fitness (Gamelin et al 2009), which may suggest that moderate exercise intensities may be more beneficial in increasing HRV in children, although it may be the training load (intensity, duration and frequency of training) that may account for the different effects on HRV (Gamelin et al 2009).

The mechanisms by which physical training exerts its beneficial effects on mortality and cardiovascular disease are surprisingly ill understood but probably include effects on blood pressure, lipid profiles, coagulation, myocyte contractile proteins, myocardial and muscular capillary density and the autonomic nervous system (Buch, Coote, and Townend 2002). Angiotensin II is known to exert inhibitory effects on the vagus nerve, with studies showing that athletes have lower levels of plasma renin activity compared to sedentary individuals (Skipka et al 1979; Fagard et al 1985; Lijnen et al 1986). Additionally exercise training increases the expression of endothelial nitric oxide (NO) synthase in coronary vasculature and neuronal nitric oxide synthase in cardiac sympathetic ganglia (Danson and Peterson 2002). Recent research has demonstrated that NO is also a modulator of cardiac vagal activity

(Buch, Coote, and Townend 2002). As this area is still largely misunderstood and a lack of studies investigating the role of NO and angiotensin II and cardiac vagal modulation, further research is required.

2.5.2 Self-assessed PA studies

One of the first studies to look at habitual PA and the HRV in adults was by Melanson (2000), with an aim of assessing day-to-day resting HRV, whether habitual PA affects reliability of measures and whether HRV is related to self-reported habitual PA levels. The study consisted of 37 male subjects aged between 25-49, each asked to complete a modified Aerobics Centre Longitudinal study of PA questionnaire reporting the number of sessions and minutes per week spent in purposeful exercise. After which, categorised into low, moderate, or high PA. HRV was recorded in the supine position for 15 minutes using an ECG allowing for time and frequency domain indices measured to be recorded. The findings of this study were 1) that HRV measures were highly reproducible, 2) time and frequency domain measures (HF and total power) were greater in active individuals (HIGH and MOD) compared to sedentary (LOW) PA although no differences in any spectral components between High and MOD, and 3) all measures in the time domain were statistically greater in active (High and MOD) versus sedentary (LOW) but no differences observed between the active individuals. Despite differences being observed between active and sedentary individuals in both time and frequency measures, habitual PA was not significantly correlated to any measure of HRV and multiple regression models indicated that self-reported weekly PA is not a predictor of HRV. Melanson (2000) reasons the data suggests that although HRV may be greater in active individuals, HRV does not appear to increase in a dose dependant manner with increasing levels of PA. The contradiction in findings may be explained in that in terms of PA, dose refers to the total energy expended in PA rather than intensity which refers to the rate of energy expenditure (Pate et al 1995), and as such the findings of the study suggest that increasing energy expenditure is not a predictor of HRV measures.

This study however has several limitations that may have affected the above results. The study included highly trained endurance athletes in the high PA category, which is not representative of the normal population, and also endurance training has been shown to increase HRV (Levy et al 1998; Stein et al 1999). Furthermore, the use of an ECG has limitations as discussed earlier in analysis of HRV (Bolanos, Nazeran, and Haltiwanger 2006).

More recently Sandercock et al (2008) investigated the differences in HRV in participants with differing habitual PA levels within the normal population range, rather than comparing athletes like in the study by Melanson (2000). This study consisted of 92 participants (47 men, 45 women; mean age 23.1±2.1 years) with habitual PA assessed by the Baecke questionnaire, and HRV indices derived from 5 minutes resting R-R interval recordings with paced breathing (0.25Hz). The results indicate that there is a positive linear relationship between habitual PA and RR interval, and more active individuals with resting bradycardia but no differences in parasympathetic modulation. The questionnaire used within this study has fair-to-moderate validity in its ability to classify individuals as low or high active but is poor at classifying moderately active individuals (Hertogh et al 2008). Sandercock et al (2008) concluded that further research using a combination of accelerometers and indices of aerobic capacity in which to classify individuals into activity tertiles is required and that objective measurement will provide data that may be regressed against RR-interval and HRV to better quantify the direction, and nature of the relationship between habitual PA and HRV.

Further studies have been conducted looking at self-reported PA and HRV both in adults and in adolescents using various questionnaires, either measuring the amount of times per session or week that an individual is sweating or hard breathing. These studies support Melanson's (2000) study in that the authors also found differences in the frequency domain and total power between the more active individuals against more sedentary participants (Dietrich et al 2008; Blom et al 2009).

The use of self-reported studies have been questioned by a review by Shephard (2003) in which the use of self-assessed PA questionnaires was found to have limited reliability and validity as a measure of PA, and as such may be argued is a major limitation within these studies. Furthermore, certain studies have used the occurrence of sweat per day/session as a measure of PA which has also been suggested as being limited in the ability to distinguish between inactive subjects in epidemiological studies (Washburn et al 1990). Limitations of self-reported PA in children involve errors from human cognitive processes, the definition of the desired variables, inadequate length of assessment and failure to account for weekdays, weekend and seasonal variations (Baronowski 1988; Cale 1994), although self-reported questionnaires is still argued as a valid and reliable measure of PA in children (Argiropoulou et al 2004).

A common feature within the literature around the use of self-reported PA is that authors tend to confuse or inter-change between physical activity and physical exercise. However, these terms are independent as PA is defined as any body movement produced by skeletal muscle that result in energy expenditure above rest, whereas physical exercise is a specific type of PA for the sole purpose of improving fitness (Trost 2001). Researchers from the American College of Sports Medicine (ACSM) found that the definition of moderate physical exercise such as "'moderate intensity' is a pace at which you break a sweat, have a slight increase in heart rate, but are still able to talk," were not sufficient in helping women understand the intensity required to meet the ACSM recommendations (ACSM 2006) further causing a possible limitation of self-report methods as the population may have different understandings of what intensity PA they had done.

2.5.3 Objective measures of PA.

With self-report studies having limitations in the measurement of PA (Washburn et al 1990; Shephard 2003), objective measures should be considered in future studies of PA and HRV as they provide a valid and reliable measure (Trost 2001; Duncan, Schofield, and Duncan 2007). There are several methods in which PA can be measured in adults and children, each with their own advantages and limitations, such as pedometers, accelerometers, double labelled water and heart rate monitoring.

The use of pedometers has become a popular monitoring tool due to their low cost and feasibility (Cardon and Bourdeaudhuij 2007) and has been to found be a reliable and valid measure of PA in adolescents and children (Rowe et al 2004; Tudor-Locke et al 2004; Eisenmann and Wickel 2005). However, pedometers do not provide the investigator with information regarding the frequency, intensity or duration of PA and pedometer steps are influenced by factors such as body size and speed of movement. Therefore, investigators should exercise caution when using pedometers in growing children (Trost 2007).

Additionally accelerometers have been used as a common method of measuring PA due to their small size, and relatively low cost (Welk, Corbin, and Dale 2000) and their ability to measure body segment movement and thus allow for the evaluation of frequency, intensity and duration of PA. There has been several validation studies involving the most commonly used accelerometers in paediatric studies (Actigraph, TriTrac and RT3 triaxial accelerometer) each concluding that accelerometers offer a valid and reliable measure of PA in children (Coleman et al 1997; Rowlands et al 2004; Freedson, Pober, and Janz 2005).

Double-labelled water is a non-obtrusive, non-invasive means to measure total daily energy expenditure in children (Trost 2007) based on the kinetics of two isotopes of water, released at different rates. This method has been found accurate within 5% to 10% (Goran 1994). However, this method is excessive in cost and also does not have the ability to provide information on the intensity of PA (Trost 2001).

Another method that was widely used in the early 90’s was heart rate monitoring, and has proved to be an reliable and valid objective assessment of PA for the most part for sustained periods of moderate and vigorous activity giving researchers a valuable insight into the nature of children’s activity (Rowlands and Eston 2007). Again though the use of heart rate monitoring to measure PA in children has its limitations, such as that due to heart rate monitors generally recording heart rate values every minute, and the lag response by heart rate to changes in movement (Rowlands et al 1997), it is suggested that heart rate monitoring may not provide a comprehensive picture of the short burst of activity and the rapid transition between activities associated with the temporal pattern of activity that is typical of children (Bailey et al 1995). Also heart rate is not only influenced by PA, it can be influenced by other parameters such as emotional stress, anxiety, level of fitness, hydration and the environment (Armstrong and Welsman 2006).

In a review examining the use of objective measurement techniques for the assessment and interpretation of PA in children, it was concluded that if the total amount of physical activity is of interest then pedometers are recommended, whilst if the intensity or the pattern of activity is of interest then accelerometry is the suggested measurement tool (Rowlands and Eston 2007). The authors of this review further suggest that because the limitations involved with both heart rate monitoring and accelerometry for the assessment of PA is not inter-correlated, a combination of the two would be more accurate than either method used alone, for example the Actiheart (Cambridge Neurotechnology, Papworth, UK) which was reported to have greater accuracy in estimating children's energy expenditure during treadmill walking and running when compared to accelerometry or heart rate alone (Corder et al 2005).

However, a search of relevant search engines (EBSCO, PubMed/MEDLINE) revealed that currently no studies have researched the relationship between pedometer measured PA and HRV in either adults or children, although a relationship between a low daily step count and coronary heart disease risk factors has been found in adolescent girls (Schofield et al 2009). Conversely accelerometers have been used as an objective measure to examine the effect of habitual on HRV. Some studies have combined self-report methods with accelerometers in middle aged men (aged 61±4) (Buchheit et al 2005) and in 12 year-old children (Buchheit et al 2007) in which it has been suggested that regular moderate activities are adequate to influence physical fitness but higher vagal related HRV indexes were found in those that took part in moderate to very high intensity PA. Furthermore, Gutin et al (2005) found favourable HRV profiles were significantly associated with higher amounts of moderate-vigorous physical activity.

3.0 Conclusion and future directions of literature

Overall the current body of literature based on habitual PA and HRV has limitations in its use of self-reported PA with the use of questionnaires, with little research assessing PA and HRV using other measuring tools such as accelerometers or pedometers. Furthermore, although there have been several studies linking PA with HRV in adults and adolescents, there is very few studies based on children. The current literature also contains confusion in the terms physical activity and physical exercise which may explain why the literature is inconclusive if some studies are suggesting it is exercise rather than physical activity that improves HRV. The findings from the previous studies investigating habitual physical activity and HRV in children concluded moderate intensity activity is significantly correlated to higher physical fitness measures, but high intensity activity is required to get favourable changes in HRV indices.

The limitations identified provide areas for further research, specifically looking at the relationship between objective measures of PA (pedometers/ accelerometers) and HRV in adults/adolescents and children. Furthermore, as research within sex and racial differences is somewhat inconsistent, future research is needed to determine whether these variables do actually impact upon HRV measures, so that it can be said that both intervention and PA studies stating improved HRV has occurred is truly independent of age, sex and racial influences.

4.0 Aims, Objectives and Hypotheses

4.1 *Aims*

The aims of the study are to assess heart rate variability, weight status and habitual physical activity in primary school children, to compare different gender, ethnicity, and age groups and weight status groups, and to investigate the relationship between habitual physical activity, heart rate variability and weight status.

4.2 *Objectives*

The objectives are:

- To measure height (cm) and weight (kg) of each child to calculate weight status groups (normal weight and overweight/obese)
- To record HRV at rest in the supine position
- To assess HRV data against age, gender, ethnicity and weight status
- To measure habitual physical activity
- Investigate a relationship between physical activity, heart rate variability and weight status

4.3 *Hypotheses*

A number of hypotheses will be examined in the present study. The experimental hypotheses are as followed:

1. Children with higher average daily step count will have an increased heart rate variability compared to those with a low average step count

2. Obese children will have a significantly lower heart rate variability in comparison to non-overweight children

3. Heart rate variability will increase significantly throughout each age group

In terms of a null hypothesis of the present study in summary is as follows;

1. There will be no significant differences in HRV with increasing age, obese children or in children with higher average step count

Chapter 2 Overview of methods

In this chapter the various methods and measurements used to assess heart rate variability and habitual physical will be discussed to provide a reference frame and rational to the methods used within the study.

1.0 Heart rate variability

1.1 Heart rate variability in children

It is important to note that although measures of short-term heart rate variability (HRV) in adults at rest have been found to be moderately reliable (Freed et al 1994; Sinnreich et al 1998), it cannot be assumed that this is the case in children. This is supported by research that concluded that the reliability of both HRV measures at rest and in light exercise have weak to moderate reliability or reproducibility (Winsley et al 2003; Leicht and Allen 2008). However, both these studies have major limitations in that a small sample size and age range were used within the studies which could have led to variations in individual HRV measures becoming significant. It is suggested that further research is required in this area because the determination of reliability provides information on the stability, accuracy and size of measurement error meaning that the data can be interpreted in a meaningful way, and the measurement tool/procedure can be replicated with confidence by others (Winsley et al 2003).

1.2 Measurement of HRV

Conventionally, HRV has been measured through the use of an ECG. However, recently it has been said to have limitations in terms of measurement and analysis from the requirement to apply algorithms to locate peak R-waves, and R-R intervals, which could lead to errors due to drift, and biologic and electromagnetic interference (Bolanos, Nazeran, and Haltiwanger 2006). Furthermore, due to the equipment not being easily portable the ECG has limitations in terms of use outside of a laboratory setting, thus leading to the development of wireless heart rate monitors. Several researchers have therefore undertaken research into the validity and reliability of such heart rate monitors (HRM) which have all concluded that specifically the Polar S810i HRM was a valid and reliable measure of R-R intervals and thus consequent HRV analysis in the supine position for adults (Gamelin, Berthoin, and Bosquet 2006; Nunan et al 2009; Weippert et al 2010) and children (Gamelin et al 2008), and also specifically the Suunto t6 HRM (Weippert et al 2010) in comparison to the ECG. Although many commercial devices offer automated measurement of HRV, the significance and meaning of the many different measures of HRV are more complex than previously appreciated causing a potential for incorrect conclusions and for excessive or unfounded extrapolations (Task force 1996).

Although the European and North American Task Force (1996) had released guidelines for the assessment of short-term HRV measures, previously little was known about the reproducibility of short-term duration HRV (Sinnreich et al 1998). When derived from stable ECG recorded under controlled, resting conditions, studies suggest that HRV is a moderately reliable measurement (Freed et al 1994; Sinnreich et al 1998; Marks and Lightfoot 1999). Within individual resting measures of HRV have been found to be reliable including; RMSSD (r = 0.20–0.98), pNN50 (r =0.43–0.97) 10 HF (r = 0.48–0.96), LF (r = 0.60–0.97), and TP (r = 0.52–0.97) (Hohnloser et al 1992; Pitzalis et al 1996; Melanson 2000). However, Sandercock et al (2005) concluded that further research is required to accurately assess the reliability of HRV following a review of current literature around measures of short-term HRV.

1.3 Position

In the supine posture, research suggests that there is increased RMSSD, pNN50 and HF, indicating parasympathetic prominence in the supine posture compared to sitting (Avbelj et al 2003; Acharya et al 2005; Zuttin et al 2008). Furthermore, there is a suggestion that there is a tendency towards higher vagal modulation in the supine position (Avbelj et al 2003). The supine position is preferable when the objective is to assess the vagal modulation of the subject's heart beat (Dantas et al 2010). In conclusion in terms of the study, the supine position is more appropriate due to investigating whether healthier/more active children have dominant parasympathetic modulation during rest.

1.4 Time

An important point to make is that the total variance of HRV increases with the length of recorded data analysed (Saul et al 1988), thus the durations of recordings need to be standardised with short-term 5 minute recordings and nominal 24-hour long-term recording being suggested as appropriate durations (Task Force 1996), although more recently 3-minute R-R series have been used to analyse HRV (Fukuba et al 2009; Weippert et al 2010). Twenty-Four hour ambulatory recordings are used to generate all-time and frequency domain measures of HRV and are more suited to analysis via spectral methods. Short-term recordings have several advantages; mainly that it is quick to perform and analyse as 5 minute recordings contain a number of individual R-R intervals that can be edited in a few minutes. Also short-term recordings can be supervised under controlled conditions by a researcher, ensuring a constant standard of measure thus reducing possible limitations. However, short-term recordings are limited in that they are unable to measure accurately very slow oscillations observed in longer recordings (Sandercock et al 2005). Therefore, in terms of assessing heart rate variability on children in a field setting, a 10 minute testing time allows for artifacts to be removed that may generate abnormal R-R interval, whilst a 3 minute analysis period is appropriate as least 1 minute is needed to assess HF components and about 2 minutes to obtain the LF component (Task Force 1996).

1.5 Controls

It is important to control the subjects' behaviour in terms of consumption of certain drinks and also physical activity prior to recording HRV. It is known that caffeine enhances parasympathetic modulation of the cardiac nervous system (Hibino et al 1997; Corti et al 2007). Furthermore, vigorous exercise 24 hours prior to testing was shown to cause an increased persistent sympathetic activation, increasing the LF component in resting conditions (Furlan et al 1993).

Another factor that may or may not be controlled is the subjects breathing, as spontaneous fluctuations in heart rate have been shown to occur in relation to the phase of respiration (Dekker et al 1997; Adams et al 2009) and an increase in sympathetic activity was shown to reduce total HRV, specifically breathing related components such as the HF frequency domain measure (Dekker et al 1997). Individuals can change their levels of respiration by increasing/decreasing their tidal volume or breathing rate subsequently altering the beat-to-beat variability of their heart rate (Adams et al 2009). Pacing respiration of an individual may not be necessary but recording the respiration and control for changes in this rate is important if not following a paced rhythm, as not doing so is a serious methodological limitation (Piche and Descarreaux 2010). However, if controlled breathing is to be used HF was found to be more reproducible when controlled breathing was used but LF more reliable under free breathing conditions (Pitzalis et al 1996). A major reduction in the power spectrum of HRV occurs between 7.5 and 15 breaths/min for HF power and between 6 and 10 breaths/min for LF power, indicating a significant low gain breathing frequency response for both LF and HF power between 15 and 24 breaths/min (Brown et al 1993).

1.6 Analysis

HRV can be analysed in either the time domain or frequency domain indices and by using non-linear parameters.

1.6.1 Time domain

There are two main groups of time domain variable measures (Stein and Kleiger 1999). The first of which include; mean R-R intervals (the differences between successive R-R intervals) and SDRR (average standard deviation of all R-R intervals), it is known that these measures reflect the circadian rhythms therefore reflects total variability (Stein and Kleiger 1999). The second group includes pNN50 (the percentage of adjacent cycles that are greater than 50ms apart in occurrence) and RMSSD (the root mean square of successive differences) which have been found to reflect vagal modulation of the SA node and correlate strongly with the high frequency (HF) power band (Pagani et al. 1986). Other time domain measurements that can be used are variations in instantaneous heart rate secondary to respiration, tilt, valsalva maneuver, or phenylephrine infusion, which can be described as either differences in heart rate or cycle length (Task Force 1996).

Most of the time domain measures correlate closely with others, thus it has been recommended that the following four measures for time domain HRV assessment should be: SDNN (estimate of overall HRV), HRV triangular index (estimate of overall HRV), SDANN (estimate of long-term components of HRV), and RMSSD (estimate of short-term components of HRV) (Task Force 1996).

1.6.2 Frequency domain

The frequency domain indices represent power density of HRV and provide information on the distribution of power as a frequency band. There are three main spectral components distinguished in a spectrum calculated from HRV recordings over a 2-5 minute period;

formed of either the high frequency band (HF) or the low frequency (LF) band or the very low frequency band (VLF) (Akselrod et al 1981; Malliani et al 1991; Task force 1996). The HF (above 0.15Hz) frequency reflects respiratory mediated HRV, while LF (from 0.04 to 0.15Hz) is modulated by both the sympathetic and parasympathetic nervous systems (Stein and Kleiger 1999). The VLF (0.003 to 0.03Hz) is influenced by the renin-angiotensin-aldosterone system, as low and respiratory frequency RR-interval rhythms, and primarily depends on the presence of parasympathetic modulation (Taylor et al 1998).

Power spectrum analysis of heart rate fluctuations provides a quantitative means of assessing the functioning of short-term cardiovascular control systems (Akselrod et al 1981). In context of short-term HRV signals, two main methods are used to determine the power spectrum, these being; Fast Fourier transformation (FFT) and the autoregressive (AR) model (Badilini, Maison-Blanche, and Coumel 1998). The spectrum resulting from the FFT is derived from all the data present in the recorded signal, whereas in the AR method components of the signal not fitting to the model is treated as noise and smoothed by partially or totally removing the data. Furthermore, it is suggested that the AR approach is more appropriate when the number of samples available for the analysis is low, because the frequency resolution of the AR-derived spectrum is not as dependent as the FFT method on the length of the recording (Parati et al 1995). It is important to note however, the spectral components of short-term HRV calculated by using the FFT and AR methods are not interchangeable (Chemla et al 2005)

In a study examining the reproducibility of the time and frequency domain measures in short-term HRV recordings it was found that all of the time domain measurements had an intra-class coefficient (ICC) of ≥ 0.75, except for the standard deviation of NN intervals, which had an ICC of 0.57. The frequency domain parameters obtained by means of either FFT or AR showed similar reproducibility. Low frequency was reproducible under all three conditions of rest, tilt and controlled respiration, total power was reproducible at rest and high frequency only during controlled respiration. Therefore, the reproducibility of the frequency domain parameters depends on the analysed condition (Pitzalis et al 1996).

2.0 Habitual physical activity

The accurate and reliable measurement of PA is necessary in determining whether PA is either an outcome measure or an intervention (Rowlands and Eston, 2007). There are several ways in which habitual physical activity (PA) can be measured, although accelerometers and pedometers have been recognised for their accuracy and validity within the literature (Cox et al 2006). Self-report questionnaires have been widely used within the literature surrounding measurement of PA due to pragmatic considerations particularly in large epidemiological studies (Freedson, Pober, and Janz 2005). However, this method of assessment does not take into consideration that children’s activity patterns are characteristically sporadic and intermittent consisting of frequent short bouts (Baquet et al 2007). Furthermore, self-report assessment has other limitations when assessing PA in that the ability for an individual to accurately recall what activity they had undertaken over a period of time is limited due to children’s levels of cognition and emotion associated with PA (Gleitman 1996). The use of pedometers has become a popular monitoring tool due to their low cost and feasibility (Cardon and Bourdeaudhuij 2007) and has been to found be a reliable and valid measure of PA in adolescents and children (Rowe et al 2004; Tudor-Locke et al 2004; Eisenmann and Wickel 2005). Pedometers are a small device that pick up movement and are widely used to assess PA (Sirard & Pate, 2001). A validation study comparing several readily available pedometers concluded that the Kenz Lifecorder (KZ), Yamax Digi-Walker SW200 (YX200), New-Lifestyles NL-2000 (NL) and the Yamax Digi-Walker SW-701 (YX701) were recommended as suitable to be applied for physical activity research (Schneider et al 2004).

However, there are several potential limitations for the use of pedometers as a measure of physical activity such as; risk of failure, risk of loss, tampering and the inability to provide information on intensity or temporal location of PA (Sirard et al 2001; Pate et al 2002). Furthermore, pedometers may result in the participants' modifying their behaviour due to the constant reminder that their PA is being assessed (Ridgers et al. 2006).

Additionally accelerometers have been found to be a reliable measure of PA, specifically the TriTrac (Professional Products, Inc., Madison, WI) in both young adults (Nichols et al 1999) and in children (Coleman et al 1997).

3.0 Methods

3.1 Participants

Following ethical approval by Coventry University Human Research Ethics Committee 303 children from a local primary school in Coventry, UK were asked to participate in the study. Assent was attained from the child along with consent from the parents/guardians, resulting in 197 children (46% boys, 54% girls, mean age 9±1.4, 87% Caucasian, 11% Asian, 2% African-Caribbean). Any children who felt ill were excused from testing, but all children were included regardless of health status i.e. children with diabetes or asthma. Testing was conducted in the main hall of the primary school during their normal timetabled physical education lessons.

3.2 Procedures

Children with returned consent all underwent assessment of weight status, HRV and habitual PA, carried out in this order.

3.2.1 Weight Status

Body height and mass were determined to the nearest 0.5cm and 0.1kg respectively using a Seca stadiometer and weighing scales (Seca Instruments Ltd, Germany). From this body mass index (BMI, kg/m^2) was determined. Weight status was classified according to the International Obesity Task Force (IOTF) criteria (Cole et al., 2000). Children were classified as normal weight and overweight/obese. The IOTF criteria was used due to being less arbitrary and more internationally based than alternatives and offers comparable prevalence rates of overweight and obese children to other countries (Cole et al 2000).

3.2.2 Heart rate variability

Participants wore a Suunto t6c memory belt heart rate monitor (Suunto Oy, Finland) for a period of 10 minutes in a supine position with arms by their sides, and legs out straight uncrossed on gymnastic mats. The first 7 minutes were used as a resting period with the final 3 minutes used for analysis (Fukuba et al 2009; Weippert et al 2010). Due to the age of children used in the study, a 10 minute testing period was thought to be more appropriate than 15 minutes in order to reduce distraction, boredom, and thus movement and talking. The 3 minute analysis time is therefore necessary to allow for a longer resting period in order to achieve a fully rested heart rate. HRV data was analysed using Kubios HRV Pro (Kubios, Finland) in both the time and frequency domain. The frequency domain was measured in both AR and FFT spectrums, because they are not interchangeable during short term analysis and offer small variations in data (Chemla et al 2005).

There was no control on variables that effect HRV such as caffeine consumption, vigorous exercise within twenty-four hours of testing and breathing rate/monitoring. As stated above, testing took place in the school during timetabled P.E lessons, therefore to control factors such as diet and exercise when the children's P.E lesson follows break-time/lunch would have proved problematic. Furthermore, the children were tested in groups of between 15-20, each of which would have slightly differing breathing/respiration rates and therefore to control breathing within a group of children would have been difficult to undertake and maintain a consistency/reliability.

A pilot study was conducted to examine the validity and reliability of the Suunto T6c memory belts used in this study, as no previous studies have investigated the reliability and validity of HRV measures against ECG recordings in children. The pilot study consisted of 14 children aged between 9-11 years old. Each subject was asked to lie in the supine position for a period of 10 minutes whilst wearing both the Suunto T6c memory belt across their chest and a 3-lead ECG monitor (PowerLab, ADinstruements) allowing for simultaneous recording of HRV. Influencing factors upon HRV such as exercise, caffeine and breathing rate were not controlled to allow for variations to occur. The final 3 minutes of data collected was used for analysis of HRV in the time domain. Recordings from the Suunto T6c memory belt and the ECG were analysed using Statistical Package for the Social Sciences (SPSS) by means of a paired t-test. The results of this pilot study showed that there was a good agreement between mean RR (r=.958), SDRR (r=.916), RMSSD (r=.934), NN50 (r=.879) and pNN50% (r=.889) and therefore suggests that the Suunto T6c memory belt is a valid measure of HRV measured in the time domain in children, when compared to measures derived from the ECG. However, the sample size of this study was small, and therefore it is suggested that a larger sample size including a range of age group should be investigated in order to confirm the findings of this study. Furthermore, frequency domain indices were not measured in this study so are unable to validate or assess the reliability of the Suunto T6c memory belt in the frequency domain in children. In conclusion the Suunto T6c memory belt offers a valid and reliable measure of HRV in the time domain in comparison to the ECG for children, and therefore an appropriate measuring tool for large studies both in lab or field settings when recording at rest.

3.2.3 Physical Activity

Yamax Digiwalker SW800 (Yamax, Japan) pedometers were the chosen method of measuring habitual physical activity due to their accuracy (within ±1% at normal walking speed) (Crouter et al 2003) and long term reliability of 0.78 when used by children (Rowe et al 2004). A four day period of measurement was used, as previous research indicates that 4-5 days of step count measurement in children results in a reliability coefficient of 0.77 (Rowe et al 2004).

Study participants were given a pedometer by trained researchers and wore it over a four day period (2 weekdays, 2 weekend days) in accordance with recommended guidelines for the assessment of habitual PA (Trost et al 2000). Participants were instructed to position their pedometer on the midline of their thigh and attach to the waist band of their clothing. Participants were then told verbally and in writing the instructions on use of the pedometers during the 4 day period, of which the principle instruction was to engage in normal activities during the measurement period. At the end of each day the participants' parents/guardians recorded the total number of steps completed for that day on their pedometer diary. Any activities that may have caused harm to the participant or breakage to the pedometer (such as rugby, and swimming), the participants were instructed to remove the pedometer and record the activity and duration. Any step count data recorded that was less than 3000 and over 40,000 were discarded, as believed to be an incorrect recording or fault of the pedometer

(Rowe et al 2004). Average step count for weekday, weekend days and over all four days were then calculated.

3.3 Statistical Analysis

Pearson product moment correlations were used to assess relationships between weight status, age and gender with HRV time and frequency domain parameters. Any differences in HRV parameters according to gender or weight status were analysed using a series of 2 (gender) X 2 (normal weight/overweight/obese) Analysis of Covariance (ANCOVA) controlling for age. Data for physical activity was assessed using a 2 (gender) X 2 (weight status) ANCOVA again controlling for age with average daily steps as the dependant variable. A series of Analysis of Variance (ANOVA) were also conducted following the same format as described for ANCOVA without controlling for age differences. SPSS (version 17) was used for all analysis and alpha level was set at priori at $P = 0.05$, and Beta values were reported following ANCOVA tests. Post hoc tests (Tukey) were used when a difference was noted to identify where the differences between the conditions and variable were.

Chapter 3 Results

Baseline characteristics of the 197 study participants by age groups are given in Table 1. Of the 197 children, 123 returned sufficient pedometer based data in order to determine average step count over a four day period due to either fault of the pedometer during the testing period, loss or the data falling outside of the recommendations made by Rowe et al 2004).

Table 1. Anthropometric data of children across age groups (mean±SD)

	Age 6 *n=5*		Age 7 *n=23*		Age 8 *n=48*		Age 9 *n=40*		Age 10 *n=42*		Age 11 *n=39*	
Gender	F 80%	M 20%	F 35%	M 65%	F 54%	M 56%	F 55%	M 45%	F 69%	M 31%	F 46%	M 54%
Height (cm)	127.7±3.6		126.8±4.4		128.9±5.1		134.5±6.2		141.2±7.1		146.2±6.8	
Weight (kg)	29.4±8.6		27.5±5.0		29.5±5.9		32.4±6.6		37.3±8.8		40.9±11.0	
BMI (kg/m^2)	18.4±4.0		17.0±3.1		17.7±3.2		17.6±2.6		18.7±3.3		18.9±3.9	

Table 1 highlights that all children were classed within the non-overweight weight status category (between the 5^{th} and 85^{th} percentile) based upon mean BMI scores, apart from children aged 6 who were classed as overweight. Individual analysis of BMI indicates that of the 197 children who participated in the study, 51 (26%) were classed as being overweight/obese according to IOTF criteria (Cole et al 2000). Aged 6 children were classed as overweight due to 3 of the 5 who participated being overweight, with 1 beyond the 95^{th} percentile thus obese.

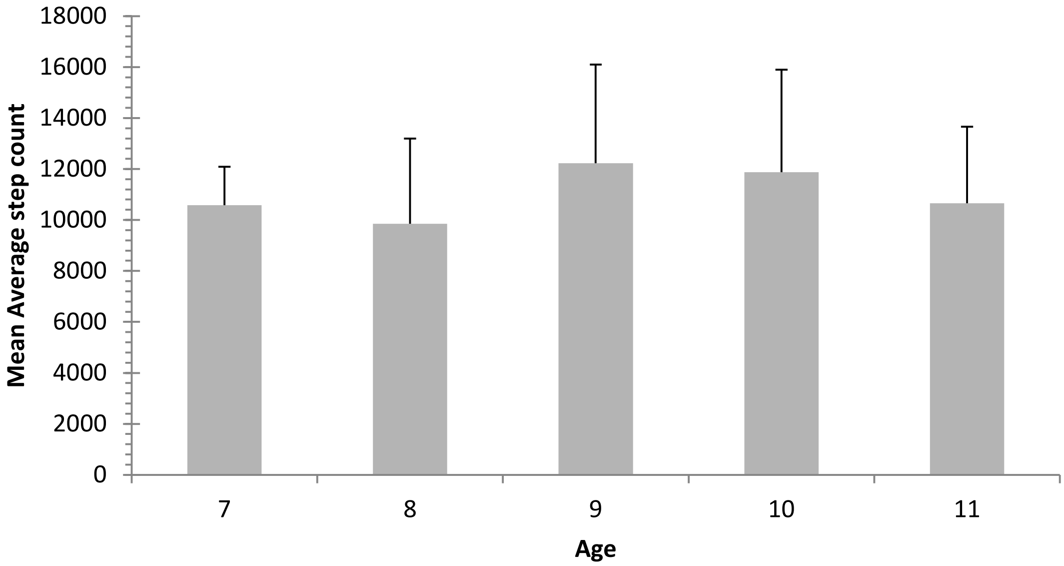


Fig. 1. Mean average step count for age groups 7 to 11.

Mean average step count for each age group is below the recommended step count for children of 16,000 steps for boys (Duncan et al 2007), although standard error/deviation indicates that the recommended daily step count for girls of 13, 000 steps is being achieved. A gradual decline in mean average step count from the age of 9 is evident in Figure 1. There is no significant difference in average step count between age groups (p>0.05). Analysis of Variance also indicated that there was no significant differences between average step count between children classified either as normal weight and those who were overweight/obese (p=.959).

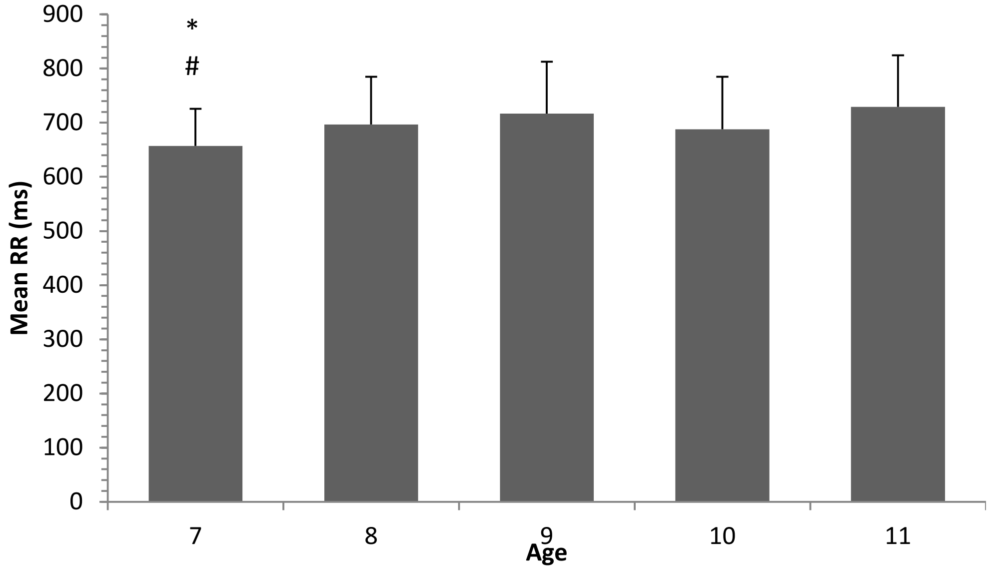
Table 2. Statistical analysis of frequency domain measures of heart rate variability variables (expressed as p values)

	FFT			AR		
	VLF	LF	HF	VLF	LF	HF
Age	0.871	0.122	0.235	0.253	0.415	0.100
Gender	0.491	0.905	0.604	0.589	0.636	0.398
Weight	0.981	0.613	0.749	0.449	0.591	0.744
Ethnicity	0.625	0.039*	0.129	0.638	0.295	0.310

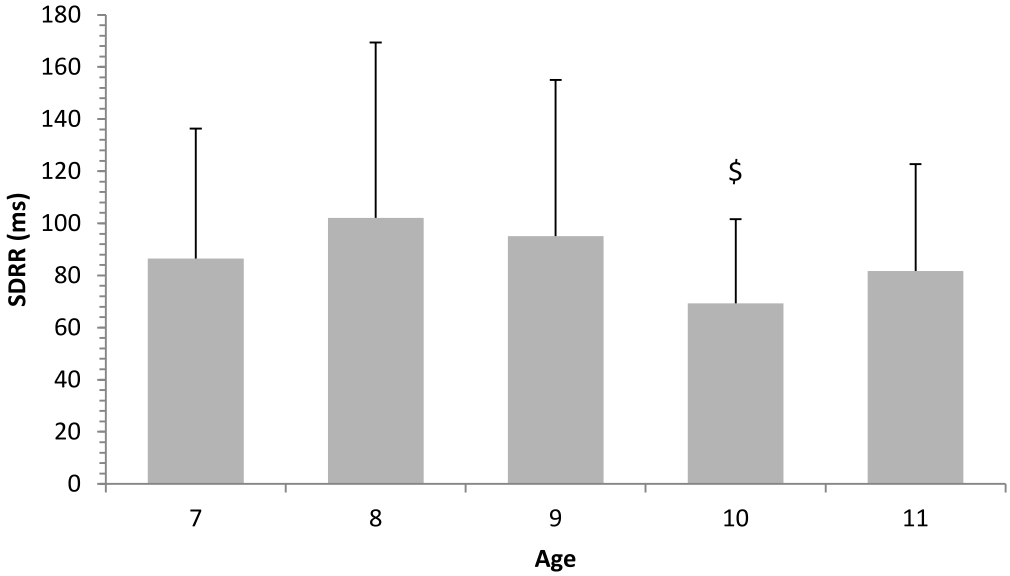
* Significant difference

Table 2 indicates that LF was found to be significant with ethnicity (P=0.039 F=3.298) in the FFT spectrum of the frequency domain. Tukey reported a difference between Caucasian children and African Caribbean children (AC) (p=0.027) and Asian children with ACs (P=0.03) for FFTLF.

a)



b)



c)

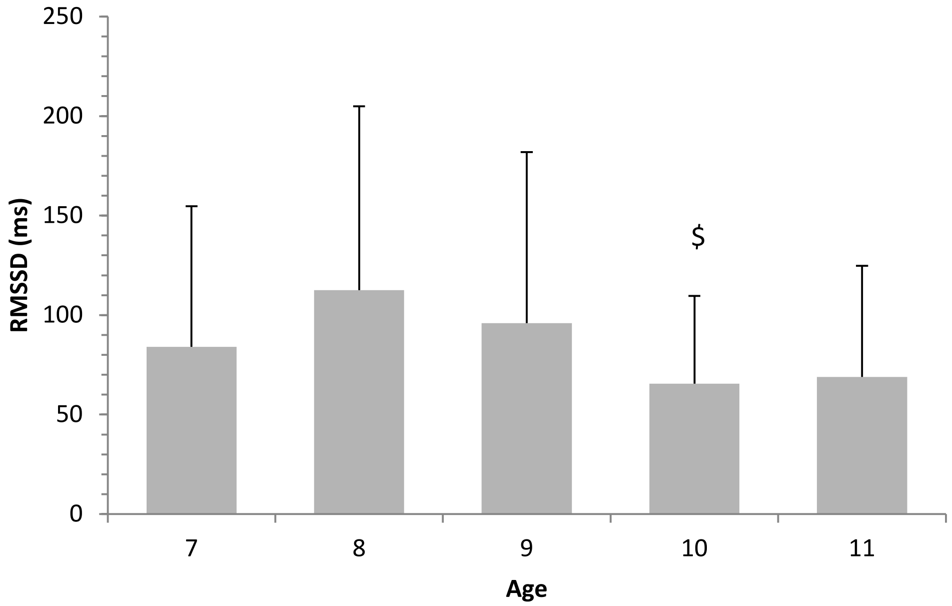
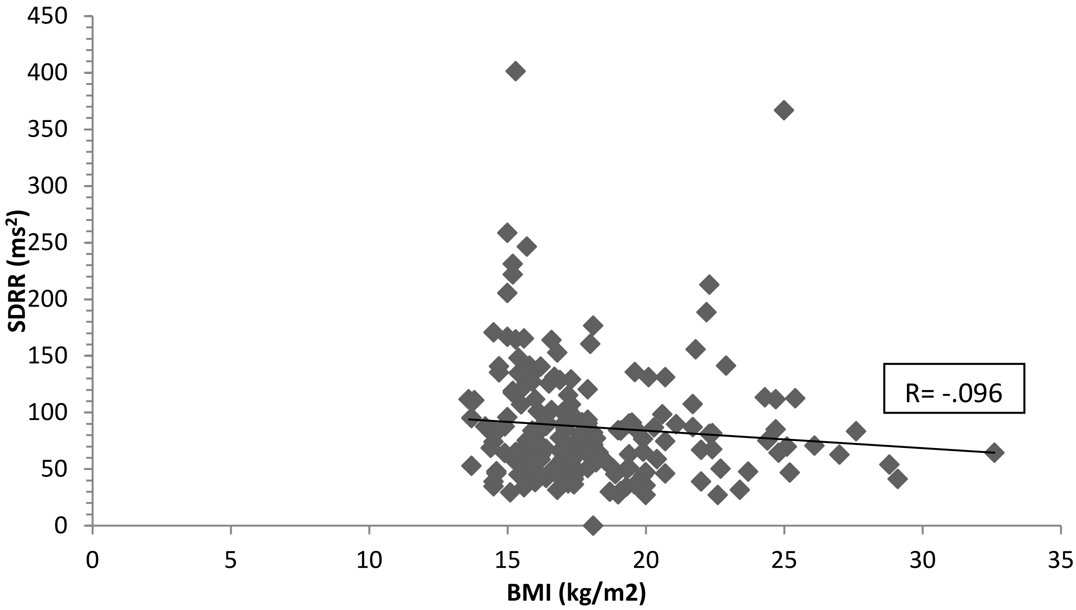


Fig. 2 Age differences for a) Mean RR intervals (* and # indicate significance between age 7 with ages 9 and 11, p>0.05) b) SDRR ($ indicates a significant difference between age 8 and 10, p>0.05) and c) RMSSD ($ indicates a significant difference age 8 and 10, p>0.05).

Fig 2 indicates a gradual increase in mean RR interval with increasing age, although there is an evident drop in mean RR for children aged 10 years old. Results from ANCOVA controlling for age indicated that there was a significant association between mean RR values and age ($F(1, 193) = 6.214$, $P = .014$, $\beta = 11.676$). Beta values indicated that each increase year increase in age was associated with an increase in mean RR of 11.676ms. Furthermore a significant association ($F(1, 192) = 3.718$, $P = .05$, $\beta = -5.222$) was found between SDRR and age, with every increase in age by one year SDRR decreases by 5.222ms. Also with RMSSD there was a significant association with age and RMSSD ($F(1, 118) = 5.417$, $P = 0.022$, $\beta = -13.517$) with every increase in age by one year, RMSSD decreases by 13.517ms. There were no significant differences with age and the frequency domain both in the FFT and AR spectrums, and with PNN50% and NN50 ($P > 0.05$). Analysis of Variance (ANOVA) was conducted to investigate significant differences between age groups, with Tukey post-hoc test used to determine where the differences lay. There was a significant difference between children aged 7 with ages 9 ($p = .05$) and 11 ($p = .011$) in mean RR but with no other age group (Figure 2a). SDRR decreases from the age of 8, with children aged 8 years old having a significantly higher ($p = .038$) SDRR than children aged 10 years (Figure 2b). As with SDRR, RMSSD decreases from the age of 8 years, with a significant decrease between children aged 8 and 10 ($p = .029$) (Figure 2c). ANOVA revealed no other significant differences between age groups in the frequency domain both in the AR and FFT spectrum, and with NN50 and PNN50%.

There were no significant differences in mean RR, SDRR, RMSSD, PNN50%, NN50, VLF and HF between gender and ethnicity groups ($P > 0.05$).

a)



b)

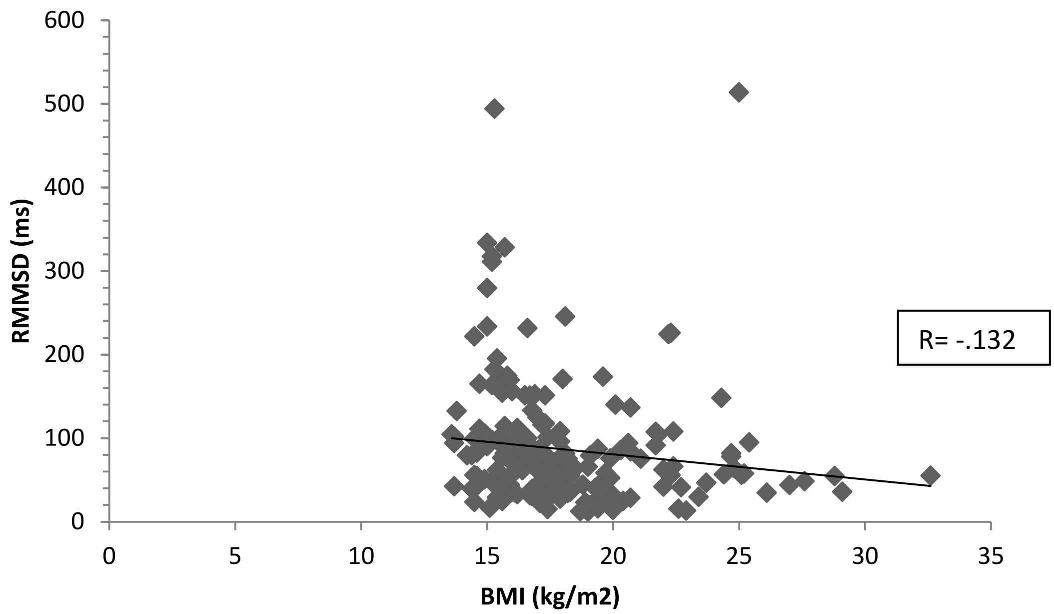


Figure 3. Correlations of individual BMI scores plotted against a) SDRR and b) RMSSD across all age groups.

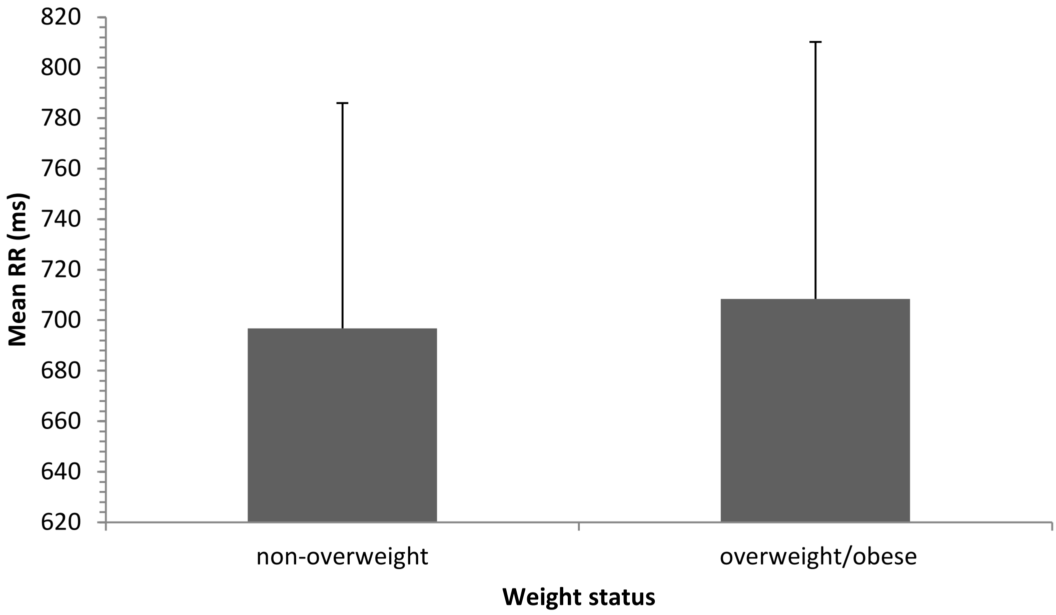


Figure 4. Average weight status differences in mean RR intervals for all age groups.

Analysis of Variance and Pearsons correlations revealed that there is no significant differences or correlations (p>0.05) between weight status groups or BMI against HRV indices (Mean RR p=.416, SDRR p=.856, RMSSD p=.261, pNN50 p=.914, FFTVLF p=.981, ARVLF p=.449, FFTLF p=.613, ARLF p=.591, FFTHF p=.749, ARHF p=.744). Although not significant (p=.064 r -.132) there is an indication towards a relationship between RMMSD and BMI. This trend is also evident for SDRR (p=.182 r-.096) (fig 3). Furthermore, Figure 4 highlights that children classified in the overweight/obese weight status category have a higher mean RR than children who are non-overweight, although this difference is not significant (p=.416) and also there is a large standard deviation present for both weight statuses.

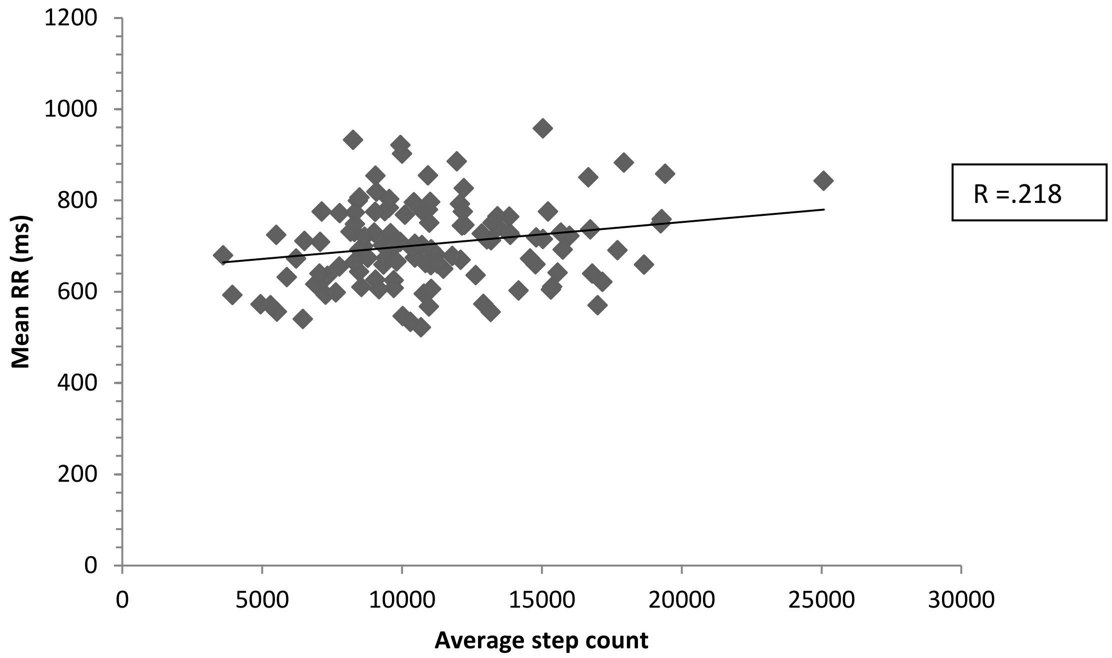


Fig. 5. Average pedometer step count over a four day period against Mean RR intervals.

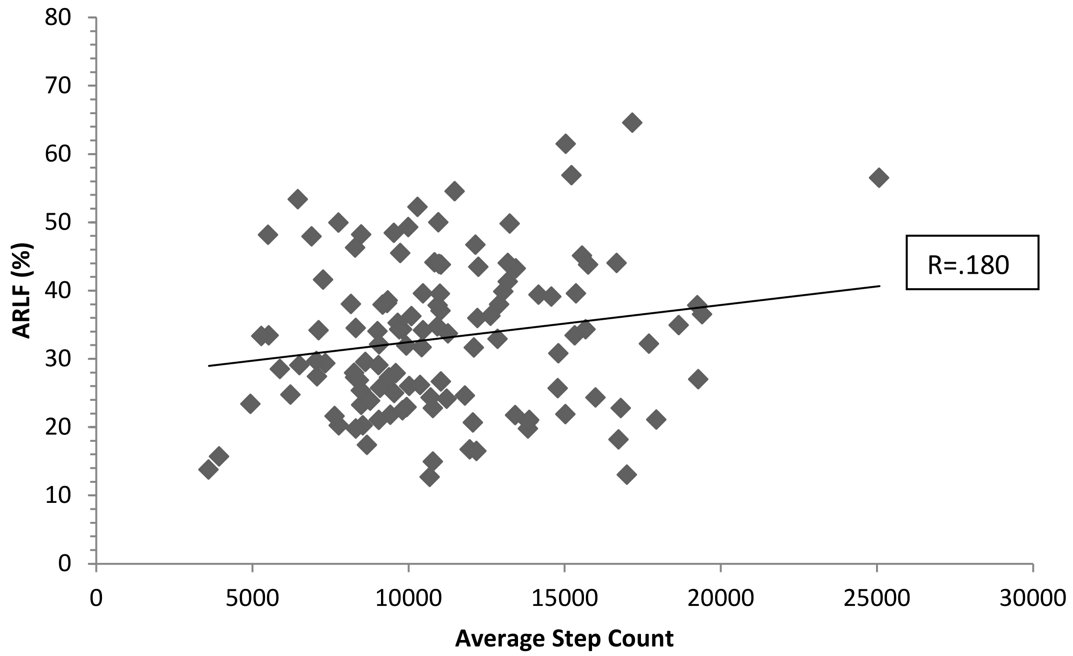


Fig. 6. Average step count and LF power in the autoregressive spectrum

Significant associations between average step count and mean RR intervals are evident ($P=0.028$, $F=5.342$, $\beta=0.005$) independent of age differences in HRV. Mean RR increases by 0.005ms with every one increase in steps, putting that in context in terms of range of average step count, there is a difference of 107.395ms between the least and most active children in terms of number of steps for RR intervals. Figure 5 indicates that there is a weak positive correlation between average steps and mean RR ($r=.218$). There was also a significant ($p=0.047$) but weak correlation ($r= 0.180$) between average step count and LF although only observed in the AR spectrum (fig 6). However, there was no other significant differences ($p>0.05$) between average step count and the remaining time domain indices nor frequency either in the FFT and AR power spectrums.

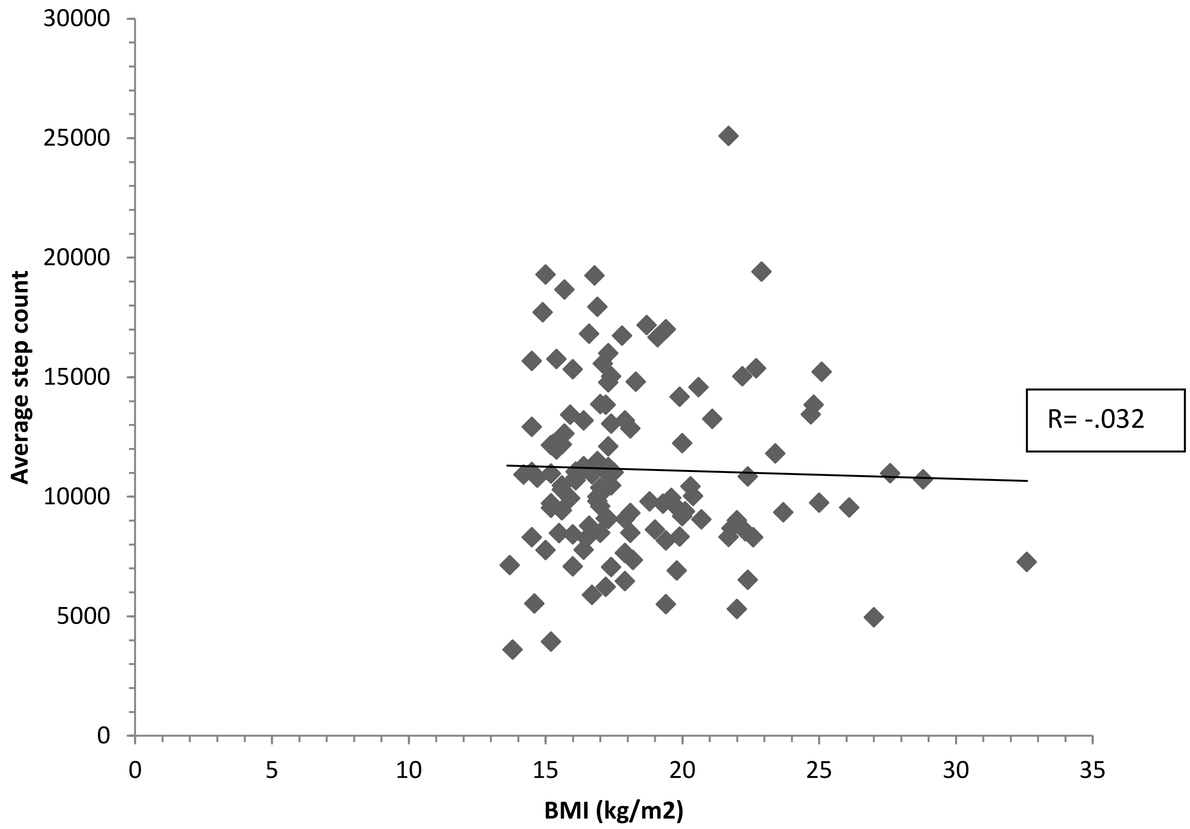


Fig. 7. Step count data over a four day period in relation to body mass index scores.

Figure 7 indicates that there is a very weak negative correlation (r-.032) between increasing BMI with average step count, although this is not significant (P=.959). Therefore, BMI does not influence the number of step an individual completes over a period of 4 days or vice versa.

Chapter 4 Discussion

The present study is the first to investigate, in children/ preadolescents, the effect of habitual physical activity on heart rate variability through the use of pedometers with the aim to examine whether step count influenced weight status and cardiac modulation inferred by HRV indices. The hypotheses of the study were 1) Children with a higher average daily step count would have an increased HRV, 2) Obese children would have a significantly lower HRV, and 3) HRV will significantly increase throughout each age group. The results of the study are three-fold: 1) Children who participate in more PA in terms of higher step count have a higher mean RR; 2) no significant weight status differences were observed in both time and frequency domain indices of HRV in children; and 3) Age differences were observed in time domain indices of Mean RR, SDRR, and RMSSD. Therefore hypotheses of the study cannot be supported in that no significant weight status differences in HRV were observed, nor were all of the HRV indices in the time and frequency domains significantly different or correlated to either average step count or age group. These findings therefore suggest that habitual PA measured by average step count is not beneficial to the increase in overall HRV in children, other than mean RR.

Previously there have only been two studies investigating habitual PA and autonomic function in children inferred by HRV, thus this study is adding to the literature base surrounding habitual PA and HRV in young children, especially as this is the first to use pedometer based PA measures with HRV.

1.1 Step count and Physical Activity

Despite a large number of studies evaluating the relationship between PA and body fat in children, it still remains unclear mainly due to the wide variety of methods used to measure

PA in children (Rowlands, Ingledew, and Eston 2000). Step count pedometers are an effective, inexpensive motivational tool in order to monitor daily activity (Sirard and Pate 2001) and thus daily step count guidelines related to positive health outcomes and promotion are essential. Current recommendations for the levels of habitual PA for children in terms of step count for reducing the risk of excess body fat in children are 16,000 for boys and 13,000 for girls (Duncan et al 2007), although more recently McCormack et al (2011) have suggested that the optimal BMI-referenced cut-off point from their sample of 7-16 year old boys and girls in Australia was 16,000 steps per day for both boys and girls.

This present study revealed that from the 123 children who returned sufficient pedometer data, only 18 girls achieved an average of the recommended step count of 13,000, and only 7 boys recorded an average step count over the four day period of 16,000 steps despite only 26% of the study participants being classified as overweight/obese. Figure 1 also shows that when the mean of the average step counts for each age group are below that of the recommended daily step count. These results may suggest that the recommended step count for children to achieve in order to reduce the risk of excessive fat is higher than required, although their study population was much larger (969 children) than that used within this present study. However, Duncan et al (2007) acknowledge that the recommendations do not account for the reduction in steps on weekend days as found previously by Rowe et al (2004) and that having guidelines for weekdays and weekend days would be overly complicated for health promotion purposes.

Vincent et al (2003) found few significant correlations between BMI and step count, these significant correlations were found in American boys aged 11 (*r*-.389) and 12 (*r*-.553) and American girls aged 9 (*r*-.364). American girls and Australian girls aged 8 years old had significant correlations of (*r*-.276) and (*r*-.331) respectively all at p<.05, although no other significant correlations were found. McCormack et al (2011) also found that healthy weight children took significantly (p<.001) more steps per day than overweight/obese boys (14,413 vs. 12,088) and girls (12,562 vs. 10,114). There is also research to suggest that it is the total accumulated time spent in vigorous PA which is related to fatness in children aged 4-6 years old - (Janz et al 2002), 5-11 (Abbott and Davies 2004), and in 8-11 year olds (Rowlands et al 1999). The positive associations observed by Vincent et al (2003), Tudor-Locke et al (2004), and McCormack et al (2011) between BMI and steps per day in predominately European communities in Australian, Swedish and American children were not replicated within this study which found that there was no significant difference (p=.959) or correlation between weight status and average step count in British primary school children (Figure 6). These findings are in agreement with Raustorp, Pangrazi, and Stahle (2004) and Duncan et al (2007) who also found no significant correlations between BMI and steps per day in Sweden and a multi-ethnic population in New Zealand respectively. Duncan et al (2007) therefore suggest that population specific step count guidelines may be warranted if the relationship between body fatness and PA does vary between countries or ethnicities, although further suggest that further research is required to determine differences, as within this study the average step count for a multi-ethnic population for British children aged from 7 years of age to 11 years, was 13,686 for boys and 10,748 for girls with a range of 21,479 steps between the least and most active individuals.

1.2 Heart rate variability and habitual physical activity

A significant positive but weak correlation (p=.028, r=.218) between average step count over a four day period and mean RR interval independent of age was found. Beta analysis indicated that with every increase in step count by one step, mean RR increased by 0.005. In context between the least and most active children in terms of step count levels there was a difference of 107.395ms, highlighting that children who achieved higher step counts had a higher mean RR than those who were less physically active. Mean RR intervals reflect the total variability of heart rate (Stein and Kleiger 1999), and indicates more active children have a lower resting heart rate with larger RR intervals highlighting a greater parasympathetic modulation of heart rate at rest than children with a lower average step count, although VLF which is influenced by parasympathetic modulation (Taylor et al 1998) was not found to be significantly correlated to average step count. However, LF which is modulated by both the sympathetic and parasympathetic nervous system (Stein and Kleiger 1999) was found to be significantly correlated to average step count ($p=.047$, $r= .180$) in the autoregressive (AR) spectrum highlighting greater parasympathetic modulation in children with a higher average step count, but this was not replicated in the Fast-Fourier transformation (FFT) spectrum offering a contradictory result suggesting no significance in either the sympathetic or parasympathetic modulation in relation to average step count. However, the AR spectrum is a more appropriate method to determine power spectrum when the number of samples available for analysis is low because the frequency resolution of the AR-derived spectrum is not dependent on the length of the recording unlike the FFT spectrum (Parati et al 1995) as in the case with this present study where only 3 minute samples were used for analysis. Therefore, it may be argued that the significant correlation between LF and average step count in the AR spectrum is more accurate. No other significant differences were observed with SDRR, RMSSD, NN50, VLF and HF ($p>0.05$). The lack of significant differences in other HRV measures and the contradictory results from the LF frequency domain suggests the association between habitual PA and RR interval appears to be modulated by a mechanism not measured by HRV (Sandercock et al 2008).

As there have not been any previous studies that have used pedometers as a measure of habitual PA when investigating the relationship with HRV either in adults or children it is hard to draw on previous literature to conclude the findings from this study. Blom et al (2009) used self-report questionnaires in 15 to 17 year old adolescents repeated over 6 months, reporting significant correlations between PA and HF, LF and SDRR although when controlled for heart rate, HF and SDRR were not significantly correlated. However, this study has limitations in its use of self-report which measured PA on a 5 point scale by the frequency of exercise with hard breathing and sweating ("never", "seldom", "once a week", "and twice a week "and "more than twice a week"). Also "hard breathing or sweating" is not defined, presumably leaving it for the subject's interpretation leading to either an over or under estimation of PA. It also does not measure for how long these periods of hard breathing or sweating take place for. The use of sweating as a measure of PA has been suggested to be limited in distinguishing active from inactive subjects in epidemiological surveys by Washburn et al (1990) thus leaves questions into the justification of the authors of these studies to use such a measure of PA. Furthermore, HRV was recorded in a sitting position rather than supine, with the supine position being suggested as preferable when assessing the vagal modulation of an individual's heart beat (Dantas et al 2010) which is more appropriate as it reflects parasympathetic modulation. Also only LF, HF and SDRR were measured in the study.

There have however, been two studies that have used accelerometry to assess PA in children in relation with HRV. Buchheit et al (2007) were the first to study preadolescent children aged 12 and found participation in activities of higher intensity for 1 hour a week was necessary to observe favourable associations with vagal related HF/LF ratios when PA was measured with a combination of accelerometry and self-report. Krishnan et al (2009) reported that increasing PA was significantly associated SDRR (p= .003, r=.356) and also RMSSD (p=.002, r= .364) but only found in boys, no significant correlations for HRV were found in girls. The findings by Buchheit et al (2007) are in agreement with Gutin et al (2005) who found moderate to vigorous PA was significant in improving vagal related HRV indices. In relation to the results from this present study, no other study in children have reported a significant correlation between PA and mean RR, although significant correlations with LF were observed by Blom et al (2009), whilst no other significant differences between PA and HRV, previous studies have reported increased SDRR and RMSSD with increasing PA in children.

It is therefore suggested that it is more the intensity of PA that a child participates in rather than the total amount of PA that is more beneficial to increasing HRV measures, of which is the main limitation in using pedometers with the inability to record the intensity of activity. As self-reported PA has limitations in its application, especially for children further research using objective measures of PA in relation to HRV in children is required to fully determine whether habitual PA is a deciding factor or whether it is more to do with regular physical exercise of a vigorous intensity for a duration of 1 hour per week.

1.3 Weight status differences in HRV

No significant differences (p>0.05) when controlling for age from ANCOVA or significant correlations from Pearson's were observed between weight status groups or BMI and HRV parameters in either the time or frequency domain. However, Figure 3 points toward a trend with very weak negative non-significant correlations between BMI with SDRR (r= -.096) and RMSSD (r= -.132), suggesting that with increased BMI children have lower SDRR and RMSSD reflecting a withdrawal in vagal modulation. Figure 4 highlights that children classified in the overweight/obese weight status category have a greater mean RR than children who are non-overweight, although this difference is not significant (P>0.05).

The area surrounding weight status differences in HRV in children has produced conflicting results so far, and the findings from this study do not completely agree with previous literature which has stated that obese children exhibit a higher sympathetic activity reflected with lower RMSSD, SDRR, and HF (Rabbai et al 2003), or alternately a reduction in both the parasympathetic and sympathetic activity has been observed in obese children (Vanderlei et al 2010). As no significant correlations were observed in the frequency domain, which indicate both sympathetic through LF and parasympathetic modulation through HF, comparison with studies that report changes in sympathetic and parasympathetic activity is not accurate. An important note to make is that the period in which a child has been obese for may be an important variable to consider when evaluating obesity and HRV (Rabbai et al 2003).

A factor that may have influenced the lack of significant correlations or differences between weight status and HRV parameters was the small number of overweight and especially obese children in each year group, for example only 9 out of 40 children were classified as overweight in children aged 11 with 7 of those being classified as obese. Significant differences between weight status and HRV parameters have mainly been reported in obese children (Karason et al 1999; Sekine et al 2001; Nagai et al 2003; Vanderlei et al 2010). There are however limitations in the use BMI for measuring adiposity in children, for example BMI is affected by stature in terms of leg length with children having higher BMI values than other children when their leg length is short relative to their height (Garn et al

1986), also BMI does not distinguish between composition in terms of fat-free mass and fat mass.

1.4 Age differences in HRV

ANOVA's revealed significant differences between age groups observed in a) Mean RR, b) SDRR, and c) RMSSD from the children who participated in this study. Children aged 7 years of age had significantly lower mean RR intervals than children aged 9 (p=.05) and aged 11 years old (p=.011). SDRR decreases from the age of 8, with a significant difference between children aged 10 years old with children aged 8 (p=.038). Again RMSSD decreases from the age of 8 in the same way as SDRR did, with a significant difference between age group 8 with children aged 10 (p=.029). Decreases in RMSSD suggest that there is development of the vagal modulation of the SA node (Pagani et al 1986), although as this is linked with the HF power band, it would have been expected for a significant correlation with age and HF but was not observed in the findings from this study. There were no other significant differences between age group for other time domain or frequency domain indices of HRV. Figure 2 also indicates wave-like changes in mean RR, SDRR, and RMSSD with a rise from children aged 7, peaking at either aged 8 or 9 before declining and rising again for children aged 11. These wave-like changes in HRV indices are consistent with previous studies which have also observed changes from year to year which may reflect the regulatory shift of adaptive character (Silvetti, Drago, and Ragonese 2001; Galev, Igisheva, and Kazin 2002).

Beta analysis from ANCOVA controlling for age revealed that mean RR significantly increases (p=.014) by 11.676ms with every one increase in year group, SDRR significantly decreases (p=.05) by 5.222ms with every increase in age by one year, and RMSSD also decreasing significantly (p=.022) by 13.517ms with every increase in age group by one year.

However, no other significant correlations between age and HRV indices in the frequency domain for both the AR and FFT spectrums or NN50 and PNN50% were observed when controlling for age. Although as mentioned by Task Force (1996) the physiological meaning of VLF derived from short recordings less than 5 minutes is questionable, and it is not recommended to interpret the meaning of the VLF parameter (Galev, Igisheva, and Kazin 2002). The increases in HRV and the decrease in heart rate through childhood have been related to the growth of the cardiac mass and stroke volume (Kmit and Rubleva 2001).

1.5 Limitations

Due to time constraints in the measurement of HRV during the children's Physical Education lessons, a 10 minute testing period was used (7 minutes rest, 3 minutes recording). Although a 3 minute period of analysis is enough time to detect both sympathetic and parasympathetic activity (Task Force 1996), and has been used in previous studies (Fukuba et al 2009; Weippert et al 2010), the recommended measurement time for HRV is over a 15 minute period with 10 minutes rest and a 5 minute analysis time (Task Force 1996). A shorter analysis period means that any variable that may affect HRV such as any slight movement or talking would be amplified greater than that if it were over 5 minutes, thus creating a possible reason to the lack of significant correlations. Furthermore, control of variables known to effect HRV such as the consumption of caffeine and exercise prior to testing were not enforced during this study due to the school's curriculum commitments which meant testing children at different times of the day meaning that, for example, children in year 6 were usually tested in the afternoon after lunch-time where they may have consumed caffeine and also took part in vigorous exercise during play time. In addition, a small ethnic range was included in this study with only 11% Asian, and 2% African-Caribbean populations.

Another important variable that may have impacted on the study's findings was that breathing/respiration rate was not controlled or monitored during the testing period for HRV, and has been suggested that not recording and controlling for changes in the respiratory rate is a serious methodological limitation (Piche and Descarreaux 2010). This is because individuals do not breathe uniformly at a fixed frequency or depth (Priban 1963). Grossman and Weintjes (1986) reported that a large percentage of breaths may occur at frequencies below 0.15Hz or above 0.33Hz and can extend above the typical RSA related to HRV. The effects of different breathing patterns on HRV however are not well established yet (Kox et al 2011). A major reduction in the power spectrum of HRV occurs between 7.5 and 15 breaths/min for HF power and between 6 and 10 breaths/min for LF power, indicating a significant low gain breathing frequency response for both LF and HF power between 15 and 24 breaths/min (Brown et al 1993). However, increased HF and a reduction in LF with increased breathing has also been described (Guzik et al 2007). Paced breathing has been reported to result in increased HF and reduced LF compared to free-breathing (Driscoll, and Dicicco 2000; Pagani et al 1991), but Pinna et al (2006) reported paced breathing does not alter autonomic regulation compared with free-breathing. In paced breathing, HF was found to be more reproducible when controlled breathing was used but LF more reliable under free breathing conditions (Pitzalis et al 1996). However, Kobayashi (2009) suggests that paced breathing provides limited improvement in reproducibility of HRV measurements, and metronome-guided respiration is not necessarily required for HRV measurement if subjects are reminded to avoid irregular respiration. This is supported by Kox et al (2011) who found that paced breathing did not affect HRV indices nor result in a significant improvement in reproducibility. It should therefore be noted that in future studies, the use of paced breathing is not necessary but the recording or monitoring of subjects breathing should be conducted whilst testing.

The majority of human activity is ambulatory, thus pedometers are a useful tool in capturing this behaviour. Furthermore pedometers were used to assess PA levels in children in this study due to their low cost, ease of application and for their overall reported validity and reliability in recording PA in children. Nonetheless, research may suggest that HRV is influenced more by the intensity of activity rather than the amount of activity an individual participates in, and also that children's activity patterns are in frequent, often vigorous short bouts. These are therefore limitations of the use of pedometers when comparing with HRV due to the inability to measure intensity, duration or frequency of activity or movement, or the temporal activity patterns unique to children. However, limitations have been identified with other measures of PA in children for example; self-report studies rely on the child's ability to accurately recall activity which is limited due to children's cognition and emotion association with PA. Accelerometers are also a commonly used, low cost measure of PA although the data is commonly expressed as a dimensionless unit which are neither meaningful of interpretable, thus requiring conversion into energy expenditure (Troiano 2006). Pedometers are therefore advantageous as the data recorded is interpretable and applicable to literature around step count, thus does not require conversion into other units. Rowlands and Eston (2007) recommended the use of a combination of accelerometry and heart rate monitoring simultaneously, for example the Actiheart which has been found to be a reliable and valid measure to assess PA. Simulated R-waves were detected within 1 bpm from 30 to 250 bpm. The 95% limits of agreement between Actiheart and ECG were found to be 4.2 to 4.3 bpm and the correlations with intensity were generally high ($r^2>0.84$, $p<0.001$) but significantly highest when combining HR and movement (SEE<1 MET) (Brage et al 2005).

Body Mass Index (BMI) was used as a measure of body fatness to classify children between normal weight and over-weight/obese children due to being a convenient measure in field settings, and the clear relationship with body fat mass in children (Goran et al 1996). Conversely, BMI has limitations in measuring adiposity in children, for example BMI is affected by stature in terms of leg length with children having higher BMI values than other children when their leg length is short relative to their height (Garn et al 1986), also BMI does not distinguish between composition in terms of fat-free mass and fat mass. Most researchers have therefore used a combination of BMI and other measures of body composition such as percentage fat when analysing weight differences.

1.6 Directions for future studies

In future studies, the measurement tool for the assessment of PA in children to investigate the correlation with HRV should consider more closely the temporal pattern of children's activity and also the intensity of activities. As this study was the first to investigate HRV in relation to habitual PA by measurement from pedometers which suggest average step count is a predictor of mean RR interval and LF in children, more research is required in this area using a larger study population to fully understand the relationship between step count and HRV. Moreover, despite numerous studies on sex, gender and ethnic differences in HRV in children, the research is inconsistent thus future research is needed to determine whether these variables do actually impact upon HRV measures, so that it can be said that both intervention and PA studies stating improved HRV has occurred is truly independent of age, sex and racial influences.

1.7 Conclusion

The hypotheses of the present study were that children with higher average daily step count will have an increased heart rate variability compared to those with a low average step count and that obese children will have a significantly lower heart rate variability in comparison to non-overweight children. Results from this study suggest that average step count is a predictor of mean RR interval in children with a significant difference between the least and most active children independent of age, although no other HRV indices were significantly correlated to average step count. No significant weight status correlations with HRV were observed, but very weak negative correlations with SDRR and RMSSD were found with BMI in children. Also weight status was not influenced by habitual physical activity measured by average step count within this study, suggesting variables other than just the total amount of physical activity influence weight status in children.

Most literature around HRV and physical activity has used self-report questionnaires which are limited in their application and analysis, especially in children due to their characteristic activity patterns and low cognition of activities. There needs to be more research into the assessment of habitual physical activity in children by objective measures that can accurately record the short, and often bouts of activity by children. Furthermore, there is a dearth of information using objective measures of physical activity and the application of this in comparison to HRV parameters, especially in children as this present study was the first to use pedometry to investigate the relationship between habitual physical activity and HRV, with only two other studies using accelerometry.

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